

## GRAFT-VS-HOST REACTION IN SKIN: HISTOLOGICAL, IMMUNOLOGICAL AND ULTRASTRUCTURAL STUDIES

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**Abstract.** A case of graft-vs-host reaction after bone marrow transplantation is described. Histopathologic and ultrastructural findings in involved skin showed necrotic keratinocytes and abnormal melanocytes with satellite lymphocytes. No deposits of immunoglobulins or complement were found. These data provide direct evidence that chronic graft-vs-host disease in humans may be related to cellular hypersensitivity rather than to serum factors.

**Key words:** Graft-vs-host reaction; Marrow transplantation; Satellite cell necrosis; Keratinocyte; Melanocyte; Direct immunofluorescence

Immunologically competent cells transferred to unresponsive recipients result in graft-vs-host reaction (GVHR) (2). Bone marrow transplanted patients with aplastic anaemia or acute leukaemia are frequently subject to such reactions (5, 10, 11). Donor lymphocytes may give rise to cellular damage in target organs, especially skin, liver and gut. Pathogenetic mechanisms are based upon the migration of mononuclear cells into epidermis. These cells behave as aggressor cells against the basal layer of epidermis (10, 14). Each dying epidermal cell is surrounded by an individual aggressor cell. This phenomenon has been termed "satellite cell necrosis" by Grogan et al. (3).

The present study reports histological, immunological and ultrastructural findings of a chronic GVHR that occurred in a bone-marrow transplanted child with aplastic anaemia.

### CASE REPORT

A 7-year-old girl with aplastic anaemia of unknown origin received high dose cyclophosphamide followed by a bone-marrow transplantation from her HL-A identical, mixed lymphocytic culture compatible sister. Mild acute GVHR occurred as an evanescent macular rash, an elevation of liver enzymes and diarrhoea. Five months

after transplantation she developed erythematous and pigmented desquamative dermatitis over her face, trunk and arms (Fig. 1) with a scleroderma-like appearance of her face. Biopsies from pathologic skin were obtained and examined by light microscopy, direct immunofluorescence (IF) microscopy, and electron microscopy.

### RESULTS

1. *Histopathology of the skin.* Biopsy material was obtained from a pigmented area of the trunk, 8 months after transplantation. Papillary dermal vessels were surrounded by a mild infiltrate of small lymphocytes. The epidermis was atrophic with hyperkeratotic plugs and suprabasilar lacunae. Those spaces were caused by degenerated keratinocytes associated with satellite lymphocytes. A few hyalinized bodies occurred without the satellitosis phenomenon, along with the degeneration of some cells of the basal layer (Fig. 2).

2. *IF findings.* The direct IF studies were done on the pigmented desquamative skin at 6 weeks and at 8 months after transplantation. These studies showed an absence of IgG, IgM, IgA, C3 and fibrinogen in the dermal vessels, at the dermal-epidermal junction, or on the surface of keratinocytes.

3. *Ultrastructural findings.* The results confirmed the presence of lymphocytic exocytosis in an atrophic epidermis. The basal cell layer was infiltrated by macrophages and small lymphocytes having dense nucleus and large nucleolus. Prominent spaces between basal cells were present. In the vicinity of basal cells, elongated lymphocytes were found with occasional close connections to adjacent dyskeratotic cells (Fig. 3). These degenerating keratinocytes showed the same characteristic features as described by Grogan et al. (3) and de Dobleer et al. (1): disappearance of desmosomes, in-



Fig. 1. Clinical lesions of graft-vs-host disease.

tracellular desmosomes and aggregated tonofilaments with subsequent acantholysis (Figs. 5–8). Various degrees of degeneration were found in keratinocytes: disappearance of cytoplasmic or-

ganelles, disruption of the nuclear membrane, and dense clumps of tonofilaments (Figs. 6–7). This suggested an irreversible process. The satellite cells presented numerous mitochondria and ribosomes and were in close association with adjacent keratinocytes without intercytoplasmic bridges (Fig. 9). Melanocytes showed numerous vacuoles containing lipid droplets and small melanosomes (Fig. 4), with satellite lymphocytes near the basement membrane.

## DISCUSSION

1. The cutaneous eruption of GVHR may be acute and characterized by an erythematous maculopapular rash involving trunk, arms, palms and soles, or chronic and manifested as dermo-epidermal sclerosis, poikilodermatous changes or exfoliative erythroderma (3). Lichen planus-like eruptions (9, 12) and toxic epidermal necrolysis (TEN)-like reactions (6, 7) may also occur. In our case, the eruption, which developed in a bone marrow transplanted girl with aplastic anaemia, was typical of chronic GVHR 8 months after the graft: scleroderma-like appearance of the face, reticulate pigmentation over the trunk (Fig. 1), hyperkeratosis and epidermal atrophy. Lichen planus-like eruption or epidermal necrolysis never occurred during the 8-month survey.

2. The characteristic histopathologic features of GVHR have been outlined by Lerner (4) as follows: grade I, vacuolar degeneration of epidermal basal cells, grade II, spongiosis and dyskeratosis (mummified bodies), grade III, bulla formation, grade IV,

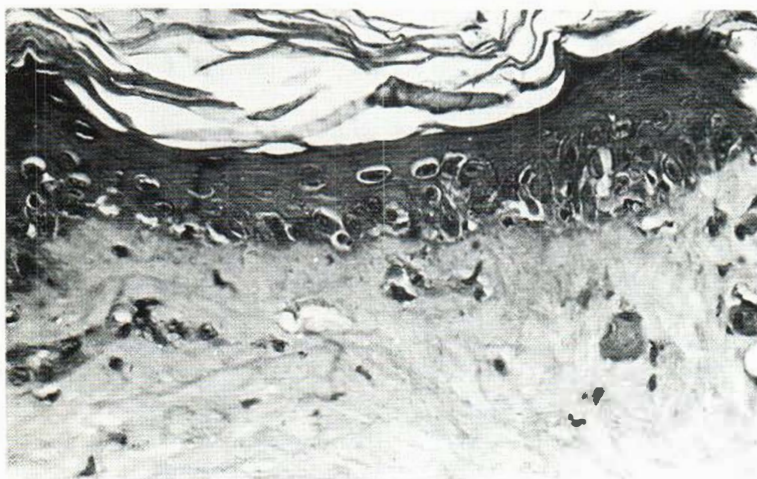


Fig. 2. Histopathology of the epidermis in involved skin.



Fig. 3. Presence of elongated lymphocyte (L) and macrophage (M) in close contact with keratinocytes (K) at the dermo-epidermal junction.  $\times 16\,000$ .

Fig. 4. Melanocyte (M) showing intracytoplasmic lipid vacuoles (G) and small-sized melanosomes (m) with satellite lymphocyte (L) near basement membrane (BM).  $\times 16\,000$ .

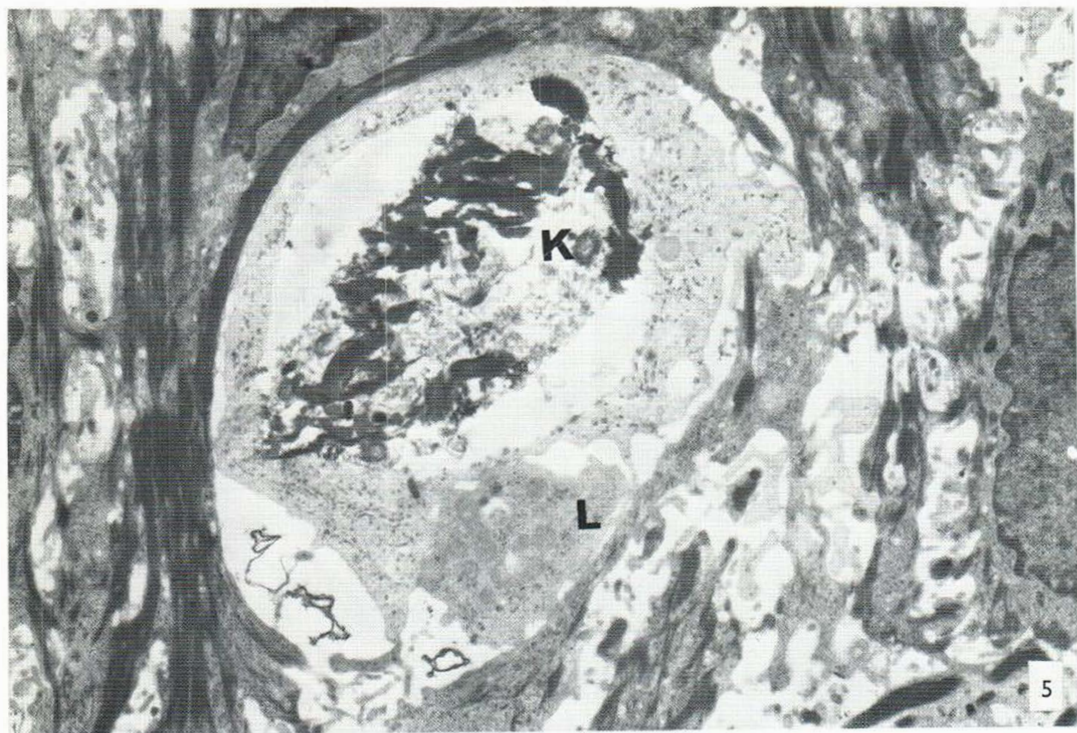


Fig. 5. Degerating keratinocyte (K) with satellite lymphocyte (L).  $\times 12\,000$ .

Fig. 6. Mummified keratinocyte with dense involuted nucleus (N), clumps of tonofilaments (t), intracytoplasmic desmosomes (D).  $\times 26\,000$ .

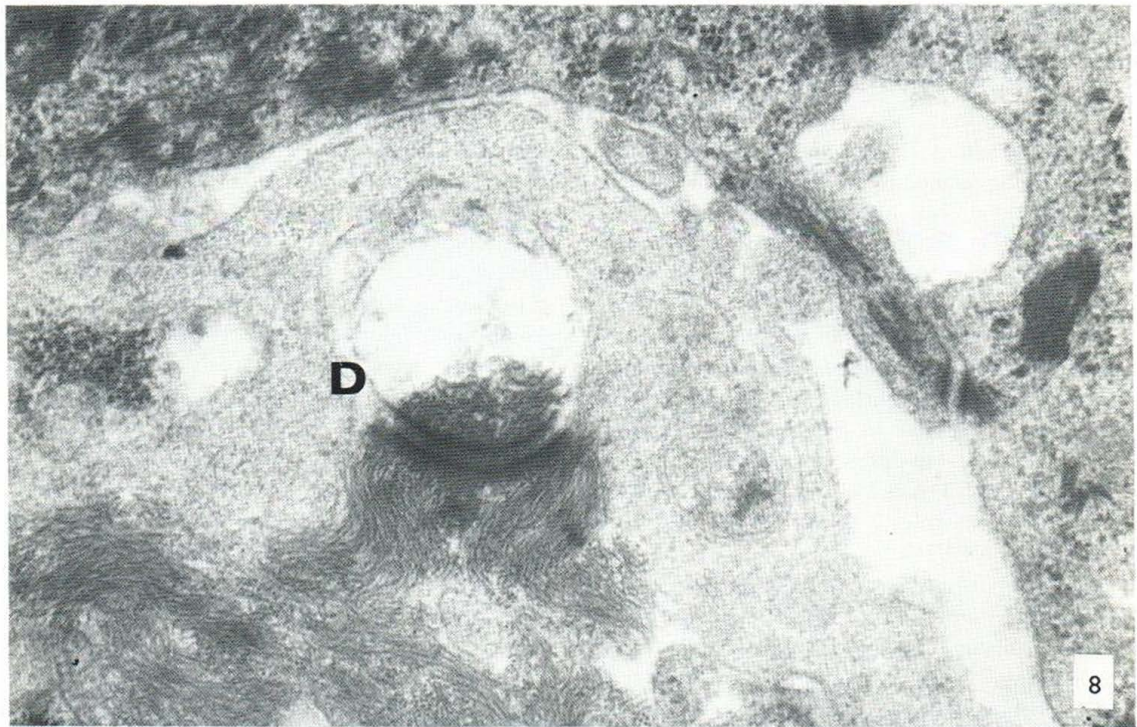
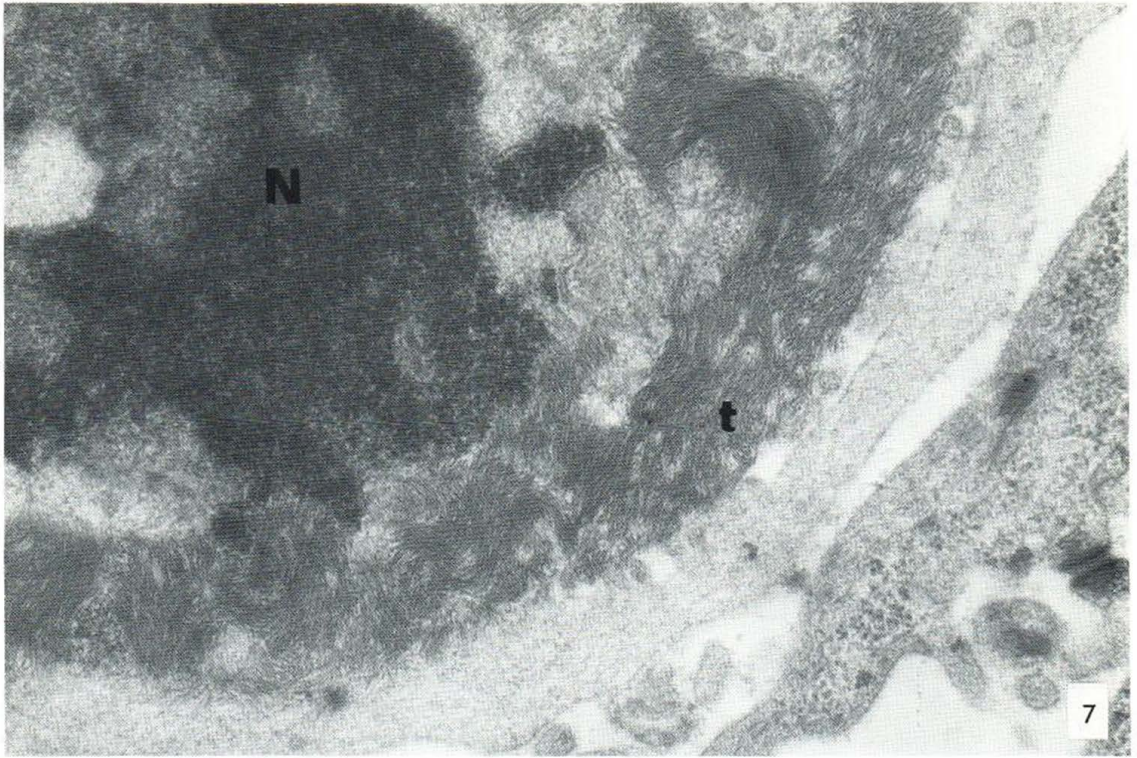


Fig. 7. Detail of involuting keratinocyte: clumps of tonofilaments (*t*) and disruption of the nuclear membrane (*N*).  $\times 66\,000$ .

Fig. 8. Intracytoplasmic desmosome (*D*) in a mummified keratinocyte.  $\times 84\,000$ .

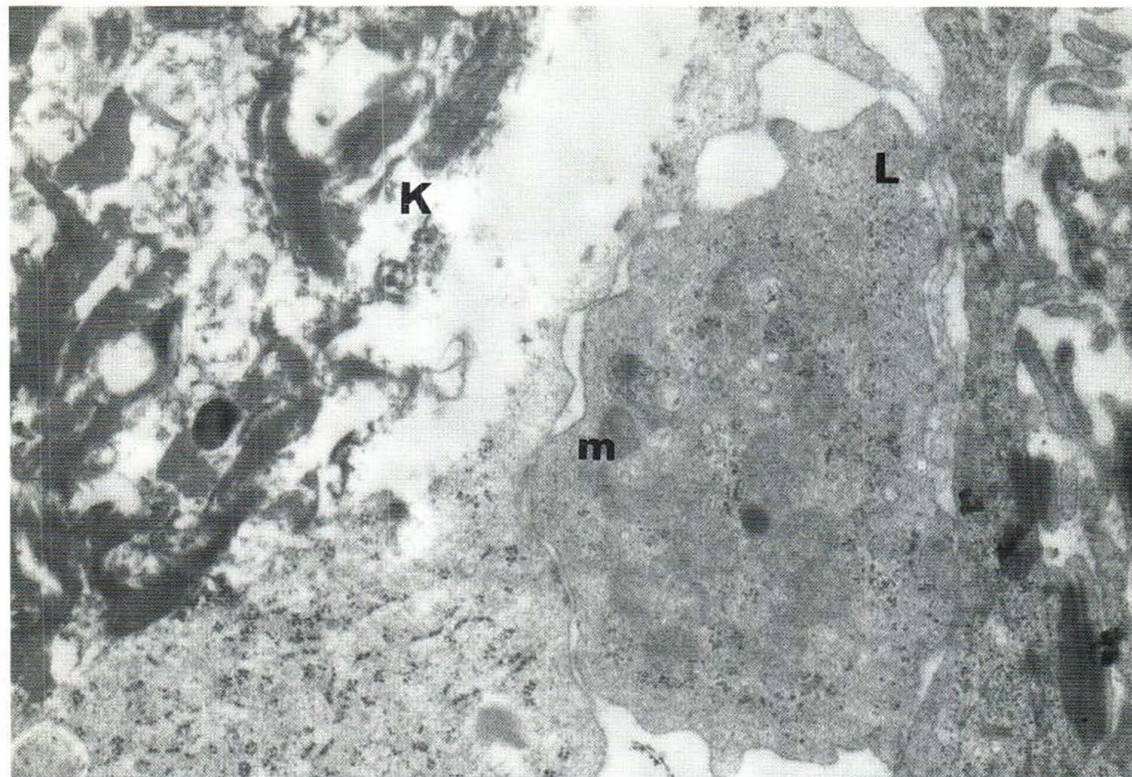


Fig. 9. Close contact of a living lymphocyte (L) and a mummified keratinocyte (K). m, mitochondria.  $\times 40\,000$ .

TEN-like reaction. These changes, although they are not pathognomonic of GVHR and may be produced by high-dose chemotherapy or irradiation given before grafting, are encountered in most allografted patients beyond week 4 (8). Our case had grade II changes.

3. The absence of immunoglobulins and complement in the vessel walls, at the dermo-epidermal junction and on the surface of keratinocytes appeared to be similar to the results obtained by Grogan et al. (3), but differed from Ullman (13) who found IgG, IgM, IgA, C3 in two patients. Thus, our results deny the role of serum factors in eliciting skin lesions in GVHR.

4. The main ultrastructural features of GVHR appeared to be the infiltration of the lower part of the epidermis by lymphocytes, accompanied by the degeneration of adjacent keratinocytes. This satellitosis phenomenon was identical to that described by Woodruff et al. (14) in experimental GVHR and by Grogan et al. (3) in man. It was not reported in de Dobbeleer's case where dyskeratotic cells and

epidermal necrolysis were interpreted as an "immunologic phenomenon complicating GVHR". The lysis phenomenon was related to the isolation of keratinocytes, endocytosis of desmosomes, disruption of nuclear membrane and disappearance of cytoplasmic organelles except for tonofilaments which formed dense clumps. These aspects resembled those seen in malignant dyskeratosis where desmosomes remain attached to aggregated tonofilaments. Although dyskeratosis is considered to be relatively non-specific, we emphasize that the satellite necrosis phenomenon seems to be distinctive enough to establish a definitive diagnosis of GVHR. Furthermore, the satellite cell necrosis suggested that cellular hypersensitivity may play a major role in eliciting the skin lesions in the absence of serum factors.

5. The presence within melanocytes of lipid-containing vacuoles in an hyperpigmented area may be interpreted as a sign of degeneration of the cell. These alterations, along with the presence of small-sized melanosomes, could be due to an accel-

erated turnover or/and to a direct effect of an adjacent lymphocyte.

The prominence and specificity of skin lesions strongly suggest that clinical and biopsy data can provide reliable early diagnosis of GVHR in man.

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Received June 13, 1978

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