Dermatomyositis: immunopathologic study of skin lesions

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ABSTRACT

Background: The pathogenesis of the inflammatory processes in the skin of dermatomyositis patients remains unclear. The aim of this study was to investigate the patterns of proliferation and apoptosis of epidermal keratinocytes and dermal infiltrating cells in DM patients.

Material and methods: Seventeen skin biopsy specimens from patients with dermatomyositis, which fulfilled the diagnostic criteria of Bohan and Peter, and Euwer and Sontheimer, were immunohistochemically investigated with monoclonal antibodies against human Ki-67, Bcl-2, CD3, CD4, anti-CD 45RO and a reaction was detected by streptovidine-biotin complex (Uni-Pak ABC).

Results: The lesional skin showed a marked increase of Ki-67-positive keratinocytes, which were predominantly located in the basal and germinative layer, but a lack of cells in areas of vacuolar degeneration and epidermal atrophy. A drastic reduction in the number of Bcl-2-positive cells localized in the basal cell compartment was observed. Antigen activated T lymphocytes (CD 45RO+) and CD3+ cells were in higher prevalence in dermal perivascular infiltrates of the affected skin.

Conclusion: The defective regulation of apoptosis may play an important role in the development of cutaneous lesions of patients with dermatomyositis, in which the skin is a prominent target organ. Abnormal expression of Ki-67 and diminution of Bcl-2 in the epidermis, coupled with the perivascular location of T cells in the dermis are crucial keys to the histological diagnosis of dermatomyositis.

KEY WORDS

dermato-myositis, skin lesions, dermato-pathology

Introduction

Dermatomyositis (DM) is an acquired multisystemic inflammatory disease of unknown origin, which affects mainly the skeletal muscles and the skin (1, 2). The pathogenesis of the inflammatory processes in DM skin lesions remains poorly understood. DM patients show increased cutaneous photosensitivity (3, 4) and the prevalence of the disease depends on geographical latitude (5). Histopathological changes of the skin in DM patients have been considered as nonspecific rewealing

poor cell interface dermatitis and dermal mucinosis, indistinguishable from those of cutaneous lupus erythematosus (LE) (6-8). Recent studies of skin biopsy specimens showed that T lymphocytes and macrophages predominate in dermal inflammatory infiltrates (9). In cutaneous lesions deposits of membrane attack complex (MAC) were found along the dermo-epidermal junction and on the vessel walls of the dermis (10). The number of apoptotic keratinocytes in the disrupted basal zone of the epidermis from DM lesions was significantly increased in comparison with normal skin (11, 12).

The aim of this study was to investigate the patterns of proliferation and apoptosis of epidermal keratinocytes and the type of infiltrating cells in the dermis of skin biopsy specimens of DM patients.

Material and methods

Patients and biopsy specimens

Seventeen skin biopsy specimens obtained from DM patients (12 women and 5 men; age varies between 20 and 81 years; average age - 58,7 years) with typical cutaneous lesions were investigated. The diagnosis was made according to the criteria established by Bohan and Peter (2) and Euwer and Sontheimer (7). Nine patients had DM as adults, four had paraneoplastic DM, three had overlap syndrome and one amyopathic DM. The morphology of cutaneous lesions, from which skin biopsy specimens were obtained was as follows: Gotron's papuls - 4, Gotron's signs - 3, heliotrope erythema – 3, erythematous plaques - 5 and poikiloderma – two samples. All biopsy samples were fixed in 10% buffered neutral formalin and paraffin embedded.

Reagents and immunohistochemistry

Five micrometer skin sections were dewaxed in xylene and rehydrated in ethanol and heated in autoclave. Endogen peroxidase activity was blocked with protein blocking reagent and sections were treated for two hours with monoclonal antibodies diluted in phosphate buffered saline in a 25^{0}C humid chamber. Proliferating cells were identified with monoclonal antibodies against Ki–67 (Biomeda corp. USA) and Bcl-2 (Oncogene - USA) antigens. Anti-CD3, anti-CD4 (DAKO A/S Denmark) and anti-CD 45RO (NOVOcasta Lab.U.K.) antibodies were used to identify T-lymphocytes and their subsets. After washing, secondary antibody reagent was added, washed again and the latter slides were developed with ABC complex (Uni-Pak ABC - Tissue Gnost® streptovidin-biotin amplified complex system) for identification of monoclonal antibody deposits. Finally, the sections were counterstained with hematoxylin and mounted. Additional sections were run in parallel, omitting the primary antibody as a negative control sample.

Scoring technique

A quantitative 5 grade score was obtained by direct microscopy including: grade 0 – no marked cells; grade 1 – 1-25% of cells; grade 2 – 26-50%; grade 3 – 51-75% and grade 4 - more than 75% of the keratinocytes and lymphocytes respectively in the epidermis and marked in the dermis. The score shows expressions of the marked cells and the percentage reveals the average of infiltrate appearance.

Results

Histopathological changes

The pathological changes of skin biopsy specimens obtained from DM patients and routinely stained with hematoxilin and eosin showed hyperkeratosis (of the knit-basket type) and vacuolar degeneration of the basal cells of epidermis, dyskeratosis and focally slightly thinned epidermis. Gottron's papules revealed acanthosis and thickened epidermal basement membrane. Oedema, perivascular lymphocytic infiltrates and dilated blood vessels were present in the dermis and the lymphocytic infiltrates appeared in hypodermic septa.

Proliferating cells with Ki-67 expression

In the epidermis of DM lesions, Ki-67 immunoreactivity was found in germinative layer (Figure 1, Table 1), but was completely absent in areas of vacuolar degeneration or epidermal atrophy. The Ki-67 nuclear antigen was also discovered in lymphocytic infiltrates in the dermis, and around the vessels and hair follicles in 23% of sections.

Proliferating cells with Bcl-2 expression

There was a drastic reduction in the number of Bcl-2-positive cells localized in the basal cell compartment of DM patients. Bcl-2+ cells rarely presented in epidermal and hair follicle keratinocytes and in only a few lymphocytes in dermal infiltrates of the specimens (Table 1).

T lymphocytes

The inflammatory lymphocyte infiltrates were usually sparse and focal in a perivascular location of the dermis (Table 2). T lymphocytes (CD3, Pan-T cells) were the most abundant cell population (Figure 2). CD3+ cells and antigen activated T cells (CD 45RO+) were in a higher prevalence in perivascular infiltrates of the papillary dermis, around the hair follicles and in a

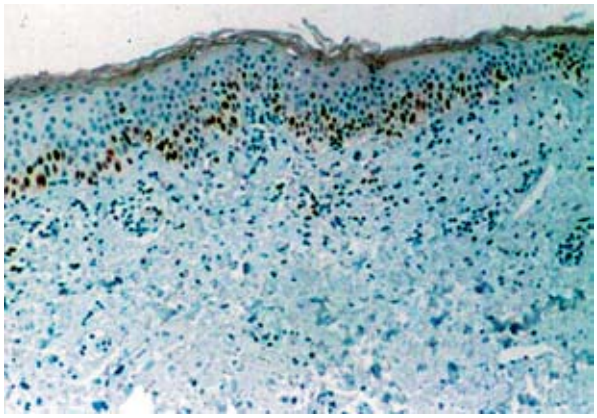

Figure 1. Dermatomyositis. Ki-67+ keratinocytes in basal and germinative layers of the epidermis demonstrated by monoclonal antibody K1-67 (x125).

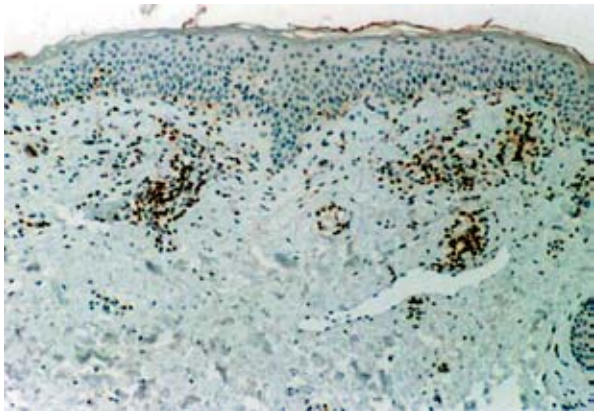

Figure 2. Dermal perivascular cell infiltrate in a patient with dermatomyositis demonstrates T lymphocytes with monoclonal antibody CD3 (x 125).

reticular dermis, than in the lichenoid "band like" infiltrate (Figure 3, 4 Table 2). CD 45RO+ lymphocytes were more frequent to be found in the papillary rather than the reticular part of the dermis and were mainly localized in the infiltrates around vessels in the skin lesions conforming to the vasculitis pattern.

Discussion

Histopathological features of skin changes in DM have been emphasized as nonspecific and similar to those found in cutaneous LE (6-9, 13). Characteristic light microscopic findings in cutaneous lesions of DM patients include: orthokeratotic hypekeratosis, partly atrophic epidermis (57%), focal basal liquefaction degeneration (69% vacuolization of the keratinocytes), melanin incontinence, papillary oedema, mucin deposit, and discrete perivascular lymphocytic infiltration (6-9, 13).

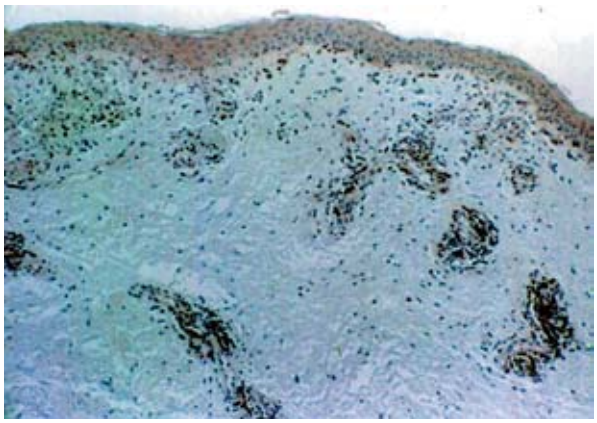

Figure 3. Dermatomyositis. Perivascular CD 45 RO+ infiltrate in papillary dermis (x 125).

In the present study of cell proliferation in DM skin lesions we found that this process is impaired in the damaged epidermis. This investigation shows a marked increase in Ki-67 positive keratinocytes, predominantly located in the basal layer of the epidermis and follicle, and a drastic reduction in the number of Bcl-2 positive cells, localized in the basal cell compartment. There have been reports on characteristic features of apoptosis in the epidermis of DM patients (11, 12). The up-regulation of adhesion molecules has also been reported in cutaneous biopsy specimens in DM patients (14). Bcl-2 is a proto-oncogene that protects permanent cells from apoptosis, encodes a 25 kD mitochondrial protein, and is localized in the 18 chromosome (15). Bcl-2 is a protein located also on the inner mitochondrial membrane, endoplasmatic reticulum and the nuclear membrane (16). It has been found that Bcl-2 is overexpressed in the serum and lymphocytes of SLE patients in comparison with SLE patients in remission and normal controls (17), as well as in the skin lesions of patients with Behçet disease (18). It has been shown that UV-A irradiation induces a fall of Bcl-2 in cell lines, and this may contribute to the induction of apoptosis, in contrast to the outcome of UV-B irradiation that damages cells through the induction of p53 (19).

The inflammatory infiltrates in DM patients have been reported as consisting predominantly of CD4 lymphocytes that are usually sparse and focal in perivascular and subepidermal locations and HLA-DR-expressing macrophages scattered through the papillary dermis, surrounding perivascular infiltrates, or in subepidermal accumulations (9). In the present study dermal infiltrates were found to consist predominantly of T lymphocytes in a perivascular location, or to have a lichenoid or periadnexal/perifollicular pattern in the der-

Table 1. Ki67 and Bcl-2 antigens in skin lesions of patients with dermatomyositis.

Infiltrate	Ki 67 Score	%	Score	Bcl-2 %
in the basal layer of the epidermis	1,7	17/17 100%	0,1	2/17 11,8%
lichenoid, perivascular and periadnexial	0,3	4/17 23,5%	0,2	2/17 17,6%

mis and septal hypodermis.

Our investigations of DM skin lesions showed the perivascular location of activated T lymphocytes (CD3+cells and CD 45RO+ cells) (Figure 3, 4). Peripheral blood T cells in adults consist mainly of CD45RO positive ones that are associated with antigen-primed memory CD8+ and CD4+ cells (20). The enrichment of perivascular T cells in the dermal compartment of DM patients supports the hypothesis that memory cells are a result of the enhanced transendothelial migratory capacity of these cells (21). These data support the concept that normal solar radiation alone or in combination with additional local mutilation factors (trauma) induces DNA damage and excessive keratinocyte apoptosis in the epidermis. The microvascular injury in the dermis allows transendothelial migration of activated T lymphocytes from the blood to the perivascular infiltrates or to infiltrates with periadnexal or perifollicular location.

Histopathologic observations on juvenile DM from the mid-1960 have suggested that the disease may represent a systemic angiopathy (22) and that the microvascular injury plays an important role in the pathogenesis of cutaneous lesions of DM (13). One of the earliest changes in DM is the focal depletion of muscle capillaries (13). Compared with LE, DM lesions showed a greater degree of endothelial injury, vascular ectasia, and vascular fibrin deposition (13). Deposits of membrane attack complex of C5b-9 complement (MAC) were found along the dermoepidermal junction in around 90% of the DM cases and in the vessel walls of the dermis in 80% of biopsy specimens (10, 13). The factors responsible for antibody deposition, complement activation and initiation of vascular damage have not yet been elucidated and the target antigen in endothelial cells remains unknown.

In contrast to the skin, inflammatory infiltrates in the muscle of DM patients were predominantly composed of B cells, T lymphocytes, and macrophages (23). Both in patients with polymyositis (PM) and DM, the muscle contained mixed infiltrates of mononuclear cells (24), but the immunological mechanisms of tissue injury are strikingly different (25). In PM patients, clonally expended autoaggressive CD8+ T cells contact and invade muscle fibers, while in DM opposite, humoral effector mechanisms prevail (24). Similar levels of perforin mRNA were expressed in PM and DM patients (25). An abundance of the CD8+ and CD4+ T cells expressing the CD45RO antigen in perivascular infiltrates of muscle specimens from DM patients was observed in all sites and accumulation of the CD45RO+ memory T cells was predominant, while the CD45RO/CD45RA ratio exceeded that in normal blood (21). The perforin in the cytoplasm of DM, was distributed randomly in the cytoplasm of muscle-infiltrating T cells, while in PM 43% of the CD8+ T cells were in contact with muscle fiber and perforin was located vectorially toward the muscle fiber (25). It has been found that T cell inflammation in PM and DM patients is not cleared by apoptosis and that the affected muscle fiber does not die as a result of apoptosis (26). Two different patterns of myofiber damage were observed in biopsies of juvenile DM, in zones with necrosis and zones where an apoptotic process predominated (27). Immunoreactivity for Bcl-2 was positive in 8 out of 10 biopsies (27). Muscle damage in DM patients is currently understood as a complement-mediated injury directed against the intramuscular microvasculature (24, 28). Several studies also have iden-

Table 2. CD 3 and CD 45RO+ cells in skin lesions of DM patients.

Infiltrate	CD3+ score	Cells %	CD 45RO score	+cells %
lichenoid	1,0	3/13	2,1	10/13 76,9%)
perivascular, in papillary dermis	2,4 ± 1,0	8/17	2,7 ± 0,6	15/17 (88,2%)
perivascular, in reticular dermis	1,7 ± 1,5	2/9	2,6 ± 0,5	7/9 (77,8%)
periadnexal/perifollicular	2,0 ± 1,4	3/10	2,1 ± 0,8	8/10 (80%)

tified MAC deposits in the blood vessels of skeletal muscle biopsy specimens obtained from DM patients and this has established the primary role of complement induced vessel injury in DM, by providing consistent evidence of activation of the complement cascade in association with capillary damage (29, 30). The serum levels of sVCAM and sE-selectin were significantly higher in the PM/DM patients than in the healthy controls (31).

The primary antigenic target in DM is the endothelium of both endomysial and skin capillaries (1). The disease begins when relevant antibodies directed against endothelial cell activate complement C3 (1). Activated C3 leads to formation of C3b, C3bNEO, and C4b fragments and finally of C5b-9 MAC, the lytic component of the complement pathway. MAC, C3b, and C4b are detected early in the patient's serum and are deposited on capillaries before inflammatory or structural changes. The complement deposits induce endothelial cell edema, vacuolization, capillary necrosis and perivascular inflammation (13). Cytokines and chemokines related to complement activation are released. They up-regulate both the vascular-cell adhesion molecule (VCAM-1) and the intercellular adhesion molecule (ICAM-1) and facilitate the egress of activated T cells not only to the perimysial and endomysial spaces but also to the dermis. T cells and macrophages through their integrins bind to the adhesion molecules and pass into the dermis through the endothelial cell wall.

The pathophysiology of photosensitive autoimmune skin diseases includes photoinduction of tumor necrosis factor alpha (TNF alpha) secretion which leads to keratinocyte apoptosis and translocation of previously sequestered cellular antigens that then activate the immune system (32). An association of the overproduction of the TNF alpha-308. A variant is found in adult DM and in subacute cutaneous LE patients (33). The TNF alpha-308 A polymorphism is associated with DM, which suggests a pathophysiological contribution from UV-induced production of TNF alpha, similar to subacute cutaneous LE. The differences in linkage with HLA-DR3, as well as several divergent clinical features, indicate that there are also fundamental mechanistic differences between DM and subacute cutaneous LE (33). In adult DM patients with one variant TNF alpha-308 A allele, at least two mannose binding lectin (MBL) polymorphisms were expressed in contrast to the controls (34). Thus, low-producing MBL genes are very strongly associated with adult DM (34). This model shows that genetic polymorphisms leading to the overproduction of apoptotic keratinocytes, and then impaired clearance of these cells contribute to the pathogenesis of adult DM, a photoinduced autoimmune skin disease (34). Immunohistochemical investigations have demonstrated that the number of apoptotic keratinocytes in the disrupted basal zone was significantly increased in patients with DM and cutaneous LE compared with normal skin (11, 12). Unlike normal skin, a large number of keratinocytes, particularly those morphologically apoptotic, expressed p53 protein. Paradoxical results have been reported concerning the induction of apoptosis in DM patients treated with corticosteroids (35). Overexpression of apoptosis in keratinocytes, muscle cells and lymphocytes has been observed in DM patients treated with corticosteroids compared with patients not given these drugs (35).

Conclusion

Our data, in agreement with that of previous studies, confirms that normal solar (UVB and UVA) irradiation alone or in combination with additional local injury induces DNA damage and excessive keratinocyte apoptosis in the epidermis of DM patients. Defective regulation of apoptosis may play an important role in the development of cutaneous lesions of DM, in which the skin is a prominent target organ. The authors believe that abnormal expression of Ki-67 and diminution of Bcl-2 in the epidermis, coupled with the perivascular location of T cells in the dermis are crucial keys in the histological diagnosis of DM.

List of abbreviations (in alphabetic order)

ABC complex - streptovidin-biotin amplified complex system
Bcl-2 - proto-oncogene, inhibitor of apoptosis
CD3 - pan-T cell antigen
CD4 - helper/inducer subtype T cell antigen
CD 45RO – reactive T cell antigen
CD8 - cytotoxic /suppressor subtype T cell antigen
C5b-9 - complement lytic complex
DM - dermatomyositis
HLA-DR – human leukocyte antigen DR locus
ICAM-1 - intercellular adhesion molecule
Ki-67 - nuclear antigen expressed in cycling cells
LE - lupus erythematosus
MAC - membrane attack complex of complement
MBL - mannose binding lectin
mRNA – mitochondrial ribonucleic acid
PM - polymyositis
p53 - tumor suppressor gene, which increases the ability of cell to undergo apoptosis
SLE - systemic lupus erythematosus
sVCAM – serum vascular-cell adhesion molecule
TNF alpha-308 A - tumor necrosis factor alpha 308 allele
UV-A - ultraviolet A rays
UV-B - ultraviolet B rays

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