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Anomalous expression of HLA class II molecules on keratinocytes and fibroblasts in hypertrophic scars consequent to thermal injury

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SUMMARY

Immunoperoxidase staining of skin sections obtained from 11 hypertrophic scars, six normotrophic scars and three samples of normal skin were performed using anti-HLA monoclonal antibodies (HLA-DR, -DQ, class I), anti-interleukin-2 receptor (IL-2R) and anti-CD1. Sections from all hypertrophic scars showed an anomalous expression of HLA-DR molecules on keratinocytes and fibroblasts. Moreover hypertrophic scars were characterized by dense infiltrates of IL-2R-positive cells and by the presence of abundant Langerhans ($CD1^+$) cells in the epidermis and dermis. These results support the hypothesis that immunologic mechanisms play an important role in hypertrophic scarring and point to an involvement of cell-mediated immune phenomena.

Keywords HLA class II hypertrophic scars fibroblasts keratinocytes

INTRODUCTION

Hypertrophic scars are the result of alterations that occur in the normal processes of cutaneous wound-healing and are usually recognized as elevated, reddish, tense, pruritic and painful masses occurring as a consequence of injury to the skin (Ketchum, Cohen & Masters, 1974; Stella *et al.*, 1989). Their incidence in any large population group remains undetermined but it is fully accepted that they are a major factor preventing many trauma patients (mainly burned persons) from recovering a satisfactory life-style (Scott McDonald & Deitch, 1987). Very little is known of the pathogenetic factors involved in this affection; both immunologic and genetic factors have been suggested (Cohen *et al.*, 1979; Janssen de Limpens & Cormane, 1982; Kisher *et al.*, 1983), but no biological pathway leading to hypertrophic scarring has been clearly demonstrated.

A characteristic feature of hypertrophic scars is a continuing production of collagen over long periods of time (Shakespeare & van Reeterghem, 1985); moreover, many histological, biochemical and immunological changes can be seen including the presence of hyperplasia, increased cell turnover and abundant leucocyte infiltrates in the dermis as well as in the epidermis (Bloch *et al.*, 1984). A common feature of cutaneous affections, characterized by the presence of relatively dense lymphocyte infiltrates (allergic contact dermatitis, mycosis fungoides, lichen planus, psoriasis), is an anomalous expression of HLA-DR antigens by keratinocytes (Volc-Platzer *et al.*, 1984; Lampert, 1984; Gottlieb *et al.*, 1986). The aberrant presence of these DR molecules has been considered as a marker of an immunologically activated state of the involved tissues (Radka, Charron & Brodsky, 1986). On this basis it seemed interesting to analyse the expression of class II molecules on the various cell types present in hypertrophic scar tissues. Frozen sections obtained from biopsies of hypertrophic scars consequent to thermal injury were analysed for the presence of HLA-DR and -DQ molecules with monoclonal antibodies (MoAbs) and the indirect immunoperoxidase technique. Normotrophic scar and normal skin biopsies were used as a control. Results demonstrate that DR molecules are strongly expressed by keratinocytes in hypertrophic scars; moreover, an aberrant expression of DR molecules was seen also on a consistent number of fibroblasts. The presence in the epidermis and dermis of hypertrophic scars of abundant activated cellular infiltrates and Langerhans cells supports the hypothesis that the abnormal expression of HLA class II molecules is the consequence or, perhaps, the responsible mechanism of an active cellular immunity.

MATERIALS AND METHODS

Patients

Biopsies were taken after informed consent from 11 patients (seven women and four men, aged 8–60 years) undergoing plastic surgery under general anaesthesia for correction of extensive hypertrophic scars consequent to thermal injury. None of the patients was treated with immunomodulating agents before surgery. Selected patients had a burned surface

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area (BSA) covering 10–40% of the body and had developed pathological scars that were still present at least 1 year after trauma and despite continuous compressive therapy. The scars were raised, erythematous, often painful, with variable degrees of skin retraction affecting articular motility; at their surface they showed telangectasies, little bullae and sometimes trophic ulcers; altogether they were judged as active lesions with no sign of regression. Hypertrophic scars were present in burned areas that healed spontaneously, in the sites where skin sections for dermo-epidermal grafts were harvested and in areas that had been primarily closed with dermo-epidermal mesh grafts.

Control specimens of normal skin were obtained from the same patients during corrective plastic surgery and normotrophic scars from patients whose lesions had an optimal healing.

Tissue specimens

Five-millimetre punch biopsies of hypertrophic ($n=11$) and normotrophic ($n=6$) scars and of normal skin ($n=3$) were obtained from different anatomical sites. Tissue samples were snap-frozen in cold isopentane (-70°C) and mounted in OCT 4583 embedding compound. Five-micron-thick cryostat sections were cut in serial sections and transferred to microscope slides. The slides were air-dried and stored at -30°C.

Monoclonal antibodies

Anti-HLA class I antibodies. MoAb W6/32 is directed against a backbone antigenic determinant on A, B, C, heavy chain associated with beta-2-microglobulin (Barnstable *et al.*, 1978); MoAb R1.30 recognizes beta-2-microglobulin.

Anti-HLA class II antibodies. HOT214 (Crepaldi *et al.*, 1985) (a gift from Dr S. Ferrone) recognizes a DR-localized monomorphic determinant also shared by DP products; AA3.84 (Malavasi *et al.*, 1984) recognizes an epitope common to DR, DQ and DP molecules. These two MoAbs are referred to as anti-DR MoAbs, although anti-DR cannot be distinguished from anti-DP reactivity. MoAb Leu10, which recognizes a frequent polymorphic epitope of DQ antigens (Chen *et al.*, 1984), was purchased from Becton Dickinson (Mountain View, CA).

Anti-interleukin-2 receptor (IL-2R) antibodies. MoAb OKT anti-Tac, was purchased from Ortho Diagnostic System, MoAb anti-CD25 from Becton Dickinson.

Anti-CD1 antibody. Langerhans cells were detected with anti-CD1 MoAb OKT6, obtained from Ortho Diagnostic System.

Indirect immunoperoxidase technique

The indirect immunoperoxidase was performed on cryostat tissue sections fixed in absolute acetone for 10 min. Serial sections were overlaid with 20 μl of different MoAbs at the appropriate dilution for 45 min. After three washings with cold phosphate-buffered saline (PBS) for 5 min, 20 μl of rabbit anti-mouse IgG (Dako, Copenhagen, Denmark) diluted 1:50 were added for 45 min; after washing, 20 μl of peroxidase–anti-peroxidase complex (Dako) were added for 60 min. Tissue cross-reaction of the second antibody was inhibited by pretreating tissue sections with rabbit normal serum. After further washings, 100 μl of substrate were added for 20 min, prepared as follows: 10 mg 3-amino-9-ethylcarbazole (AEC, Sigma) were dissolved in 6 ml DMSO and diluted to 1 mM concentration in 0·02 M sodium acetate buffer/0·15 M NaCl+0·003% hydrogen peroxide. Endogenous peroxidase activity was inhibited by addition of methyl alcohol and 0·03% hydrogen peroxide. All samples were counter-stained with Mayer's haemalum solution, and slides were examined under a microscope. Slides were examined double blindly.

Table 1. Different expression of HLA class I and class II antigens in hypertrophic scars, normotrophic scars and normal skin

Tissue	Cell type	A, B, C	DR	DQ	IL-2R
Hypertrophic scars	Keratinocytes,	+	+	±	±
($n=11$)	infiltrating	+	+	+	+
	endothelia,	+	+	+	−
	fibroblasts	+	+	−	−
Normotrophic scars	Keratinocytes,	+	−	−	−
($n=6$)	infiltrating	+	+	−	−
	endothelia,	+	+	+	−
	fibroblasts	+	−	−	−
Normal skin	Keratinocytes,	+	−	−	−
($n=3$)	infiltrating	N	N	N	N
	endothelia,	+	+	+	−
	fibroblasts	+	−	−	−

HLA-A, B, C was detected with monoclonal antibodies (MoAbs) W6/32 and R1.30; HLA-DR+DP with AA3.84 and HOT 214; HLA-DQ with Leu 10; interleukin-2 receptor (IL-2R) was detected with OKT anti-Tac anti-CD25.

Scale: +, detectable on all samples; −, undetectable; ±, detectable on some samples; and N, in normal skin, infiltrating cells were not detected.

RESULTS

Skin sections from 11 patients with hypertrophic scars, secondary to thermal injury, and from six patients with normotrophic scars and three biopsies of normal skin as a control, were stained by MoAbs with an indirect immunoperoxidase technique. The reactivity of anti-HLA-DR (HOT214 and AA3.84), anti-HLA-DQ (Leu10), anti-HLA class I (W6/32 and R1.30), anti-IL-2R (OKT anti-Tac and anti-CD25), anti-CD1 (OKT6) MoAbs were tested on serial sections. The results obtained (Table 1) were practically identical in all the specimens of hypertrophic scars, irrespective of the anatomical site and the extension of the lesion.

Epidermis

Keratinocytes in skin sections from all the hypertrophic scars were strongly stained with anti-HLA-DR MoAbs (Fig. 1a). In five specimens where glandular ducts were present, glandular epithelia also appeared to express DR molecules. In the controls, as expected, keratinocytes and glandular epithelia were negative (Fig. 1c).

The expression of HLA-DQ molecules was also analysed: only in one hypertrophic scar sample there was a faint positivity present on all keratinocytes, while in all the other samples the epithelial cells were negative and the only cells stained with MoAb Leu10 were Langerhans cells.

The IL-2R was detectable on keratinocytes in one biopsy out of the 11 tested and was distributed in foci. The same pattern was shown by OKT anti-Tac and anti-CD25 MoAbs.

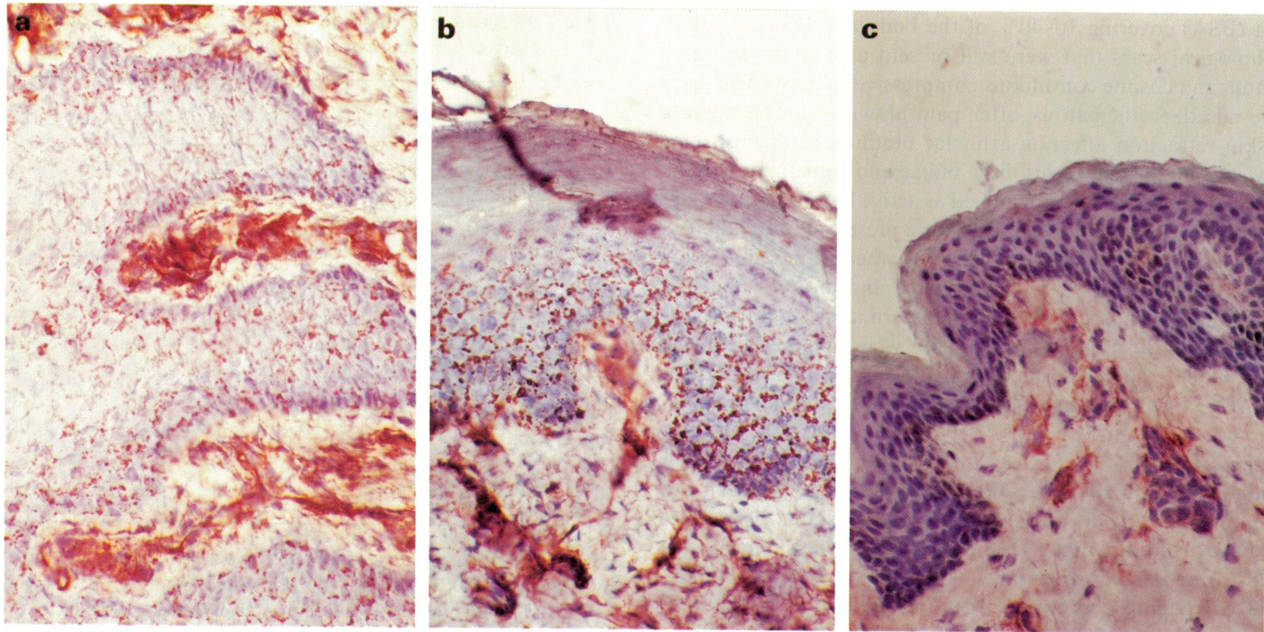

Fig. 1. Immunoperoxidase staining of sections of epidermis (a) hypertrophic scar with anti-DR MoAb (HOT214), keratinocytes show intense membrane reactivity; (b) hypertrophic scar with anti-class I MoAb (W6/32), keratinocytes are positive; (c) normotrophic scar with anti-DR MoAb (HOT214), keratinocytes are negative. Magnification ×140.

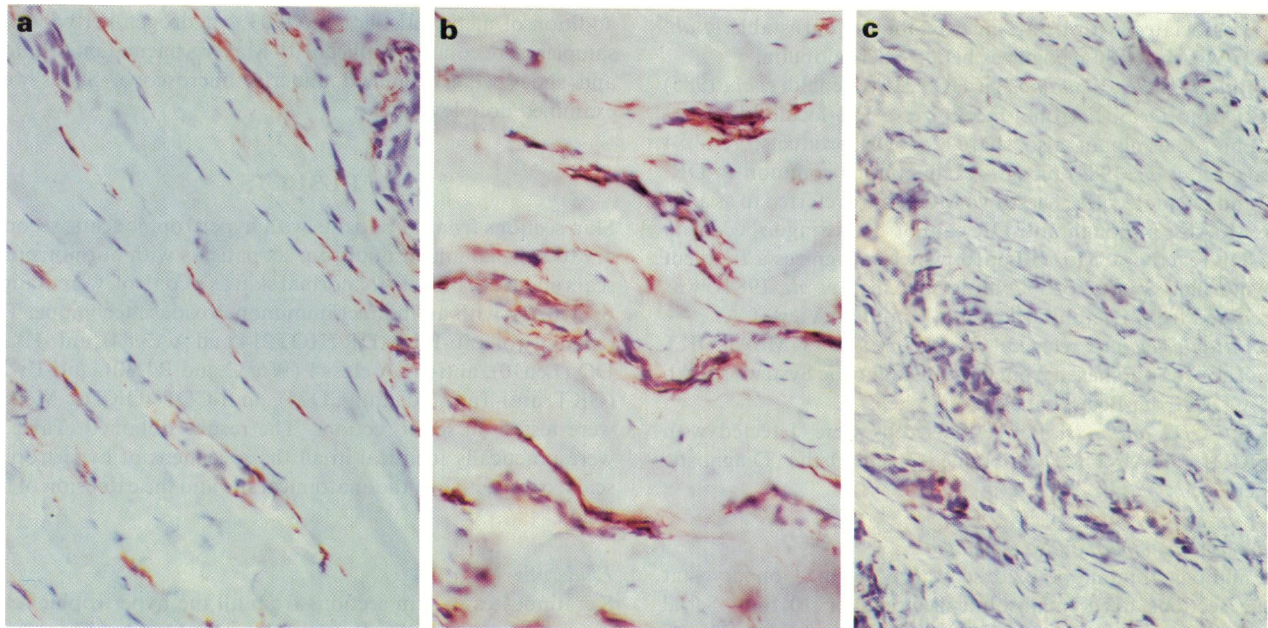

Fig. 2. Immunoperoxidase staining of sections of dermis from hypertrophic scars with (a) anti-DR MoAb HOT 214, about 30% fibroblasts are very strongly stained; magnification ×140; (b) anti-DR MoAb HOT214; magnification ×280; (c) anti-DQ MoAb Leu 10, fibroblasts are negative; magnification ×140.

A great number of OKT6-positive cells (Langerhans cells) were detected in all biopsies from hypertrophic scars. Langerhans cells were also present in controls but in a lower amount. In controls, Langerhans cells were the only DR- and DQ-positive cells detectable in the epidermis.

Class I antigens were expressed on epithelial cells in all biopsies (Fig. 1b).

Dermis

The endothelia of large and capillary vessels were always stained with anti-class I and anti-DR MoAbs; anti-DQ MoAb showed a more restricted positivity. An aberrant expression of HLA-DR molecules on a consistent number of fibroblasts (about 30%, see Fig. 2a, b) was clearly detected in all sections of hypertrophic scars. DR-positive fibroblasts could be found mostly in the

deeper dermis with infiltrations around blood vessels; DQ molecules were not detectable on the same fibroblasts (Fig. 2c). HLA-DR and HLA-DQ antigens were never seen on fibroblasts in normotrophic scars and in normal skin.

Epidermal and dermal infiltrates

The serial sections of hypertrophic scars were characterized by the presence of abundant cellular infiltrates that could be seen as clusters of few cells in the epidermis and as dense agglomerates localized close to the endothelial cells of the vessels in the papillary (Fig. 1a, b) and in the deeper dermis. Both epidermal and dermal infiltrates were in all the samples strongly stained with anti-DR and to a lesser extent also with anti-DQ and anti-IL-2R MoAbs. Since the presence of B lymphocytes in dermal and epidermal infiltrates is unlikely, the above pattern was probably due to activated T cells and macrophages. Clusters of Langerhans cells (OKT6-positive) were present in all dermal infiltrates of hypertrophic sections analysed. Cellular infiltrates ubiquitously expressed class I molecules. In normotrophic scars, infiltrates were less dense than in hypertrophic scars and were sometimes scarcely detectable; when present, they were positively stained with anti-class I and anti-class II MoAbs but not with anti-IL-2R. In normal skin, dermal infiltrates were absent.

DISCUSSION

Several publications underline the immunological aspects of hypertrophic scars, such as the increased tissue IgG deposition compared with normal scars (Cohen *et al.*, 1979; Kisher *et al.*, 1983), the suggestion that individuals with high levels of circulating immunoglobulins are more likely to produce post-traumatic hypertrophic scars than those with lower values (Bloch *et al.*, 1984), the presence of autoantibodies against collagen matrix (Quismorio, Bland & Friou, 1971) and the increased number of Langerhans cells in the scars (Cracco *et al.*, 1989). The presence of DR molecules on fibroblasts and on keratinocytes demonstrated here emphasizes the role that immune mechanisms play in hypertrophic scarring and point to the involvement of cell-mediated immune phenomena.

An analogous positive expression of DR molecules has been previously reported on keratinocytes of psoriatic plaques and in some other dermatological affections (Volc-Platzer *et al.*, 1984; Lampert, 1984; Gottlieb *et al.*, 1986). The use of anti-DR+DP MoAbs in the present study does not allow us to distinguish between the two types of class II molecules, although it is likely that DR molecules are expressed at a higher level than DP (Natali *et al.*, 1986). DQ molecules were detected only in one biopsy: this result is in accord with data indicating that in general induction in constitutively class II-negative cells seems to be preferentially exerted on DR and DP rather than DQ molecules (Natali *et al.*, 1984; Lecchi *et al.*, 1989). DQ might be present on keratinocytes at a level which is not detectable by the methods used in this investigation. The anomalous presence of class II molecules on fibroblasts may be related to the active role of these cells in the development of scar tissue, since they actively synthesize collagen (Shakespeare & van Reeterghem, 1985) during the repair process of skin injury. At present it is not known whether the expression of DR molecules on non-immunocompetent cells (keratinocytes or/and fibroblasts) converts the same cells into effective antigen-presenting cells in dermatological affections. Data are available concerning this aspect in some autoimmune diseases: it has been demonstrated that cultured thyroid epithelial cells from patients with Graves' disease expressing class II molecules could successfully present influenza haemagglutinin peptides to a T cell clone (Londei *et al.*, 1984).

It is likely that in the case of human keratinocytes, the induction of HLA-DR antigen is not the essential primary event in triggering an immune response (Czernielewski, 1985) but is secondary to increased levels of interferon-gamma found as part of a generalized release of T lymphocyte-derived lymphokines during lympho-epidermal interaction (Czernielewski & Bagot, 1986). The presence of IL-2R-positive infiltrates is in line with this hypothesis. The question remains whether aberrant expression of class II molecules can be considered as an immunological alteration capable of initiating autoimmune response or just an activation marker that can be found in inflammatory autoimmune processes without being of primary importance in triggering autoimmunity. Moreover, some data (Wick, Hala & Wolf, 1986) suggest that expression of DR molecules may not be primarily induced by 'environmental factors,' but is secondary to the specific autoimmune assault. In fact, infiltration by mononuclear cells seems to be a prerequisite for the aberrant expression of DR antigens.

Irrespective of the answer to these questions, our results indicate that therapy of the scar could be aimed usefully at the control of cell-mediated immunity.

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