

Monochromatic excimer light (308 nm): an immunohistochemical study of cutaneous T cells and apoptosis-related molecules in psoriasis

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ABSTRACT

Background Various types of UVB radiation source (290–320 nm) are used in treating psoriasis and their therapeutic mechanism has been attributed to immunosuppressive properties. Recently, a new UVB source generated by a 308-nm excimer laser has been introduced for the treatment of psoriasis.

Objective In this study we investigated the immunohistochemical evaluation of T cells and the expression of various apoptosis-related molecules in the psoriatic hyperproliferative skin before and after treatment with 308-nm monochromatic excimer light (MEL).

Methods Ten patients (three women and seven men), ranging in age from 29 to 79 years, affected by plaque-type psoriasis vulgaris, were treated with MEL. Biopsies from psoriatic lesions of MEL-treated sites were taken before, 24 h and/or 48 h after the first irradiation and analysed by the immunophosphatase alkaline technique (APAAP).

Results MEL treatment was found to cause a significant decrease in the rate of proliferation of keratinocytes and a relevant depletion of T cells in all psoriatic lesions, 48 h after the first irradiation: 308 nm light eliminated T cells from the psoriatic epidermis and also from the dermis, highlighting the ability of this UVB source to penetrate the skin compared with normal UVB and establish direct cytotoxic action on T cells infiltrating skin lesions. Rapid clearing of psoriatic lesions involves potential molecular targets of UVB in T cells including p53, which is upregulated after direct irradiation with 308-nm UVB. Moreover, Bcl-2 expression in healing psoriasis epidermis after MEL treatment is significantly decreased compared with untreated skin and the TUNEL (TdT-mediated dUTP-biotin nick end labelling) technique revealed the presence of relevant apoptotic keratinocytes in the irradiated epidermis.

Conclusions These results indicate that psoriatic skin after monochromatic excimer light therapy is associated with significant T-cell depletion and alterations of apoptosis-related molecules accompanied by a decreased proliferation index and clinical remission.

Key words: psoriasis, apoptosis, 308 nm UVB, Ki-67, p53, Bcl-2, TUNEL, immunosuppression, T lymphocyte

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Introduction

Psoriasis is a common inflammatory and hyperproliferative skin disease characterized by hyperproliferation of keratinocytes and the presence of acute and chronic inflammatory cells.^{1,2} This marked thickening of the epidermis might be related to the imbalance of the homeostasis caused by abnormal apoptotic process.³

Several features of this disease suggest an immune mechanism mediated by activated T cells infiltrating into the

epidermis associated with dermal accumulation of T lymphocytes with a predominant helper CD4+ pattern.⁴

Various types of UVB radiation source (290–320 nm) are used to treat psoriasis and their therapeutic mechanism has been attributed to immunosuppressive properties.⁵

Recently, in addition to narrow-band UVB (NB-UVB) (311 nm), which is more effective than older systems,^{6,7} a new UVB source generated by a 308-nm excimer laser has been introduced for the treatment of psoriasis.^{8–10}

In the present study we investigated the immunohistochemical evaluation of T cells and the expression of various apoptosis-related molecules in psoriatic skin before and after treatment with a 308-nm monochromatic excimer light (MEL). The aim was to verify whether the clearance of psoriatic plaque produced by the effect of this new UVB source might be due to the depletion of T cells and involve apoptotic mechanisms.

Materials and methods

Patients

Ten subjects (three females, seven males) aged 29–79 years (mean age 51.5 years) and affected by plaque-type psoriasis vulgaris were recruited, following a wash-out period of at least 6 weeks prior to enrolment. All participants gave their informed consent to perform the study.

MEL treatment

The minimal erythema dose (MED) for each patient was established on uninvolved dorsal skin before irradiation with a new type of XeCl lamp able to generate non-coherent, monochromatic 308-nm UVB radiation (EXCILITE-DEKA, Florence, Italy) that produces a power density of 50 mW/cm² at a distance of 15 cm from the source and a maximum irradiating area of 512 cm². After protecting uninvolved skin, each psoriatic area was irradiated starting from the MED time (range 8–15 s) and increasing by 3–10 s during subsequent applications to a maximum of 90 s. The irradiation sessions were performed three times at week and the treatment was stopped upon attaining clinical resolution of psoriatic lesions.

Immunohistochemical study

After informed consent skin punch biopsies were taken, under local anaesthesia, from psoriatic lesions of MEL-treated sites before the start of MEL treatment and 24 and/or 48 h after the first irradiation. When lesions appeared to be clinically cleared, another biopsy was obtained from the same area.

Biopsies were snap-frozen in tissue-tek (OCT solution) with liquid nitrogen and stored at –80 °C until they were prepared for the immunophosphatase alkaline technique (APAAP) and the detection of apoptosis by TUNEL (TdT-mediated dUTP-biotin nick end labelling) with the *In situ* Apoptosis Detection Kit (Alexis Biochemicals, San Diego, CA, USA).

APAAP procedure

Sequential cryostat sections (5 µm) were cut from each specimen, air dried and fixed in 100% acetone. Sections were washed in TRIS-buffered saline, coated with Protein Block (DAKO, Glostrup, Denmark) for 10 min, washed again and

then incubated for 60 min with monoclonal antibodies to Ki-67 (1 : 30), CD3 (1 : 20), CD4 (1 : 20), CD8 (1 : 20), p53 (1 : 50) and Bcl-2 (1 : 50) (all supplied by DAKO, Glostrup, Denmark).

Sections incubated with a non-relevant isotype-matched mouse antibody were used as negative controls. After washing for 10 min, sections were incubated for 40 min with rabbit antimouse Ig antiserum (RAM, 1 : 30; DAKO, Glostrup, Denmark) and then processed with alkaline phosphatase antialkaline phosphatase complex (APAAP, 1 : 50; DAKO, Glostrup, Denmark). In order to enhance the labelling intensity, incubation with RAM and APAAP were repeated for an additional cycle. Binding of the complex was revealed by fuchsin as chromogenic substrate (Merck, Darmstadt, Germany). Sections were then counterstained with Mayers haematoxylin, cleared and mounted in Resin-Based Permanent Mounting Medium (Immunotech, Marseille Cedex, France). Two independent and 'blinded' observers evaluated serial sections. For quantitative analysis, the stained cells were counted in three consecutive microscopic fields (× 200), in both the epidermis and the dermis, summed, and the average was then calculated.

Statistical analysis

Results were expressed as mean ± standard deviation (SD) of the mean. Statistical significance ($P < 0.05$) was assessed by Student's *t*-test.

Results

Epidermal proliferation

In all samples examined, the Ki-67 signal was exclusively confined to the nuclei with no cytoplasmic staining being observed. In psoriatic skin, the Ki-67 positive cells were observed mainly in the basal and suprabasal cell layers (fig. 1a) and this pattern of Ki-67 expression was topographically similar to that of lesions treated with 308-nm UVB light (fig. 1b).

However, the epidermal proliferation in UVB-treated psoriatic lesions showed a significantly lower mean Ki-67 index of treated skin than that of untreated skin ($P < 0.001$).

Infiltrating cells

The immunohistochemical analysis revealed a significant reduction in infiltrating cells in psoriatic patients after treatment with MEL (Table 1).

In particular, fig. 2 shows that the ability of 308-nm UVB to deplete CD3 and CD4 T cells in the psoriatic skin of all cases was observed 24 h after treatment (fig 2b and 2e, respectively), and after 48 h the CD3+ (fig. 2c) and CD4+ (fig. 2f) reductions in both the dermis and the epidermis were also greater and very significant.

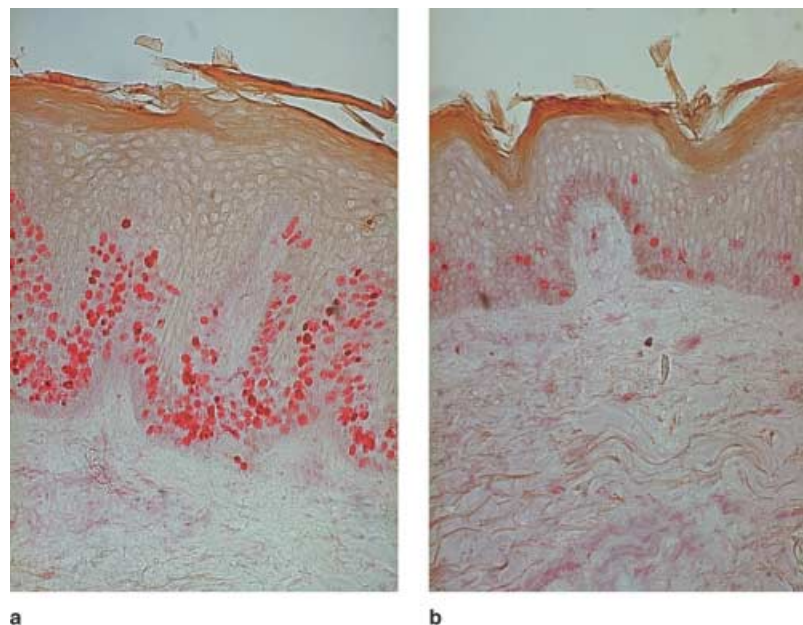


fig. 1 Expression of Ki-67 in psoriatic lesional skin before (a) and after treatment (b) with 308-nm excimer light (original magnification $\times 200$).

Table 1 T cell markers detected by immunohistochemistry pre- and post-treatment with 308-nm excimer UVB

	Pre-treatment	Post 24 h	Post 48 h	Remission
<i>Dermis</i>				
CD3	172.8 \pm 81.0	152.0 \pm 75.1	71.2 \pm 57.4**	45.7 \pm 23.4**
CD4	231.7 \pm 71.8	215.8 \pm 63.1	108.8 \pm 64.2**	96.0 \pm 21.3**
CD8	50.4 \pm 28.4	75.0 \pm 17.9*	46.3 \pm 48.4	17.8 \pm 13.7**
<i>Epidermis</i>				
CD3	52.6 \pm 40.8	32.6 \pm 13.8*	26.6 \pm 36.0*	2.7 \pm 3.4**
CD4	56.7 \pm 37.1	42.9 \pm 17.8	20.8 \pm 24.5*	8.3 \pm 5.8**
CD8	20.0 \pm 19.9	25.2 \pm 19.8	15.0 \pm 22.3	1.3 \pm 1.3**

All values are expressed as mean \pm standard deviation; *0.001 $< P < 0.05$; ** $P < 0.001$ by Student's *t*-test.

At the end of treatment all patients showed a very significant decrease in the expression of the infiltrating cells present in the superficial dermis, while a very small number of lymphocytes was detected in the epidermis with a scattered distribution. A marked increase in the expression of CD8+ cells that is significant in the dermis was observed 24 h after treatment (fig. 2h). These cells were mainly distributed around papillary and superficial vascular dermal sites. At the end of treatment CD8+ cells were also extremely reduced in the patient's skin (fig. 2i).

Immunohistochemical analysis of apoptosis in established and healing psoriasis post-MEL

In pretreated psoriatic plaques immunoreactive to anti-p53 antibody, which is a regulatory factor inducing apoptosis, was observed but only moderate staining was present in the dermis while the epidermis was always negative (fig. 3a). When the skin was irradiated with 308-nm UVB light, the percentage of

p53-positive infiltrating cells increased rapidly (fig. 3b): there were 13.43 (SD = 9.33) p53 positive cells per field before treatment, 23.77 (SD = 7.83; 0.001 $< P < 0.05$) after 24 h and 17.66 (SD = 8.5) after 48 h. At remission, the expression of p53 evaluated in the study showed lower levels compared to those initial (10 \pm 3.92; $P < 0.05$) p53 values. Positive and intense staining for Bcl-2, an anti-apoptotic protein, was detected before the treatment on basal keratinocytes, and on the cellular infiltrate localized in the papillary dermis and near the dermo-epidermal junction. Twenty-four and 48 h following the excimer treatment the cell count did not show statistically significant differences, while after the trial patient specimens showed an evident reduced epidermal staining for this molecule and a moderate and focal staining on infiltrating dermal cells ($P < 0.001$). Figure 4 shows analysis of Bcl-2 positive cells in lesioned epidermis from a psoriatic patient before (fig. 4a) and after (fig. 4b) irradiation with 308-nm UVB.

Detection of apoptotic keratinocytes

In order to detect apoptotic keratinocytes, we performed immunohistochemical analysis using TUNEL antibody, which reacts with apoptotic cells with DNA fragmentation.

Apoptotic cells were marginally detected in psoriatic untreated epidermis (fig. 5a).

In contrast, the psoriatic MEL-irradiated epidermis showed marked and slightly increased apoptotic cells (fig. 5b,c).

Discussion

Skin irradiation with ultraviolet UVB light (290–320 nm) is a common and often effective treatment for psoriasis and other

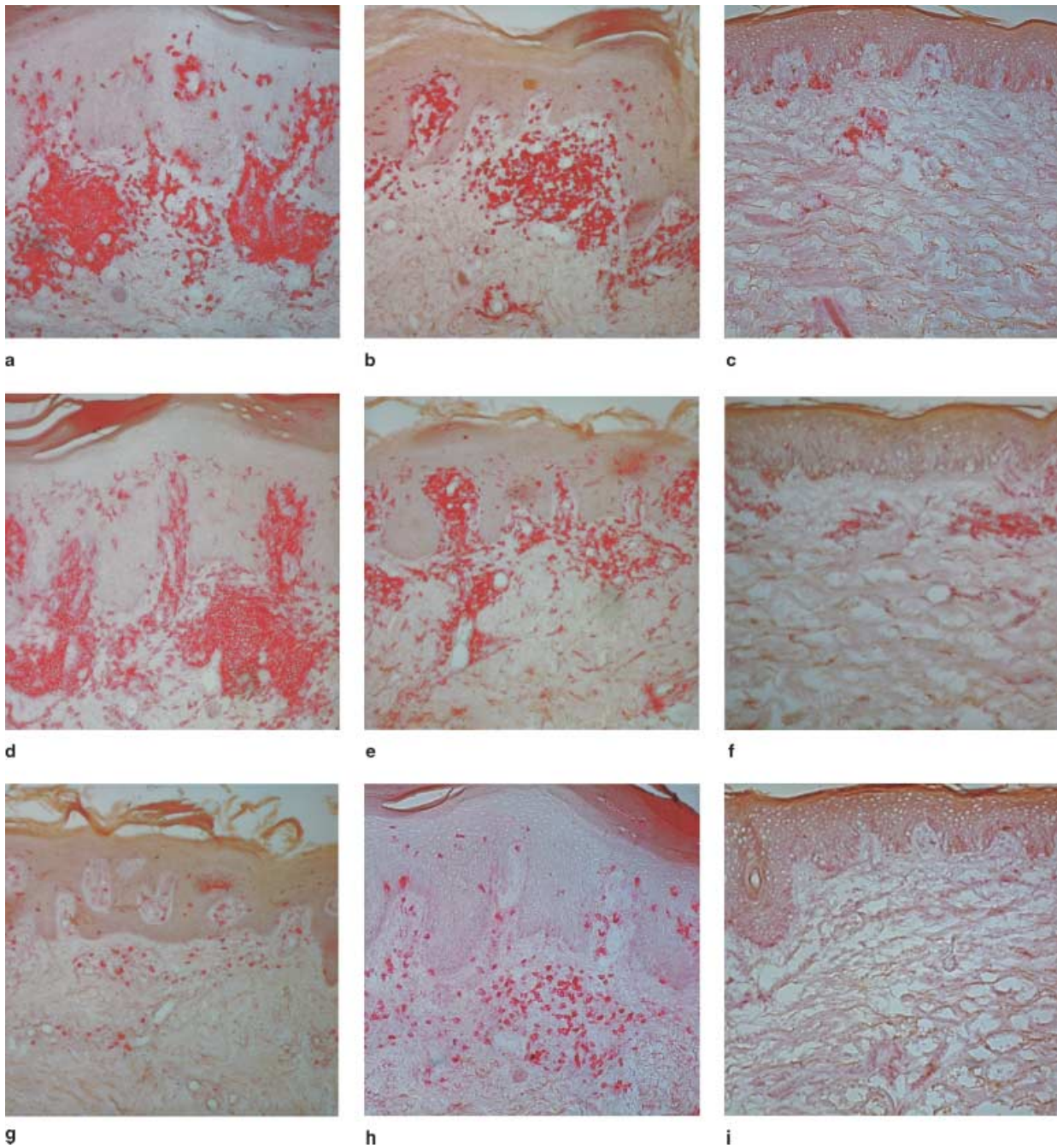


fig. 2 Immunohistochemical detection of T cells in psoriatic lesional skin before and after 24 or 48 h of irradiation with 308 nm excimer light. *Left*, untreated psoriatic lesions (a,d,g); *middle*, 24 h 308-nm UVB-treated lesions (b,e,h); *right*, 48-h 308-nm UVB-treated lesions (c,f,i); *top*, CD3+ cells (a,b,c); *centre*, CD4+ cells (d,e,f); *bottom* CD8+ cells (g,h,i); (original magnification $\times 100$). Panels show markedly decreased T lymphocyte expression in treated psoriatic skin.

inflammatory skin disorders, but irradiation with standard UVB light also produces a sunburn reaction (intense erythema and keratinocyte necrosis). It has been demonstrated that the wavelengths from 290 to 300 nm have erythemogenic effects but no therapeutic benefits¹¹ whereas phototherapy by MEL at 308 nm, a wavelength near the 311-nm narrow-band UVB, showed high effectiveness without adverse reactions.¹²

Experimental evidence suggests that UVB radiation produces many immunosuppressive effects on the skin^{13,14} and particularly that the mechanism of NB-UVB action in inflammatory skin disorders involves cytotoxic effects on T lymphocyte cells.¹⁵

In this study we carried out an immunohistochemical examination of the effects of MEL treatment on lymphocytes in psoriasis.

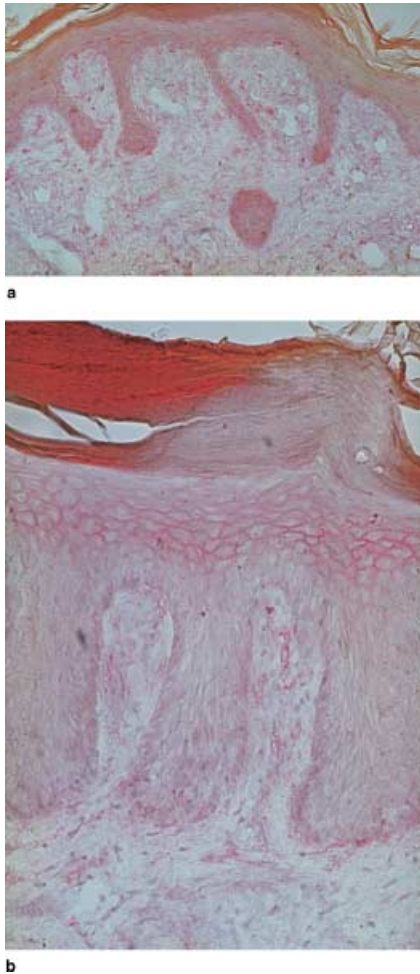


fig. 3 Expression of p53 in untreated (a) and 24 h treated (b) psoriatic lesions (original magnification $\times 200$).

Most of the cellular and histopathological changes that typify the psoriatic epidermis reverted to normal in all patients treated.

Our results show that MEL treatment causes a significant depletion of T cells in all psoriatic lesions, 48 h after the first

irradiation: 308 nm light eliminated T cells from the psoriatic epidermis as well as from the dermis, suggesting the enhanced ability of this UVB radiation to penetrate the skin in comparison with normal UVB⁵ and to establish direct cytotoxic action on T cells infiltrating skin lesions. This result is in agreement with the drastically decreasing cytokine expression demonstrated by Cappugi *et al.*¹⁶

Rapid clearing of psoriatic lesions could involve potential molecular targets of UVB in T cells including p53, which is upregulated or stabilized after direct irradiation with UVB and could potentially mediate apoptosis.^{17,18} In fact, rapid apoptosis was produced in isolated leucocytes by small amounts of UVB *in vitro*.^{5,19}

Another protein playing a key role in cell homeostasis is the protooncogene Bcl-2, which is negatively regulated by p53.²⁰ Psoriasis can be viewed as a hyperproliferative disorder of keratinocytes mediated by T cells. One possible explanation is that proteins associated with preventing apoptosis (Bcl-2, Bcl-xL) are overexpressed on keratinocytes within lesional plaques,²¹ increasing epidermal thickness in psoriasis.

In the present study we showed that Bcl-2 was expressed in basal to spinous cell layers: the expression level of Bcl-2 in the healing psoriasis epidermis after MEL treatment was significantly decreased compared with the untreated skin. In this regard, loss of Bcl-2 expression in complex epithelia has been shown to correlate with differentiation and loss of proliferation.²²

To further investigate the possibility that apoptosis could be an essential factor in the regression of psoriasis after 308-nm UVB therapy, we investigated the TUNEL-based apoptotic index. Our analysis revealed no evidence for apoptosis in pre-treated psoriatic skin, whereas the biopsies made 48 h after treatment documented TUNEL positive cells throughout all cell layers of the epidermis.

In summary, these results indicate that psoriatic skin after monochromatic excimer light therapy is associated with significant T cell depletion and alterations in apoptosis-related molecules, accompanied by a decreased proliferation index and by clinical remission.

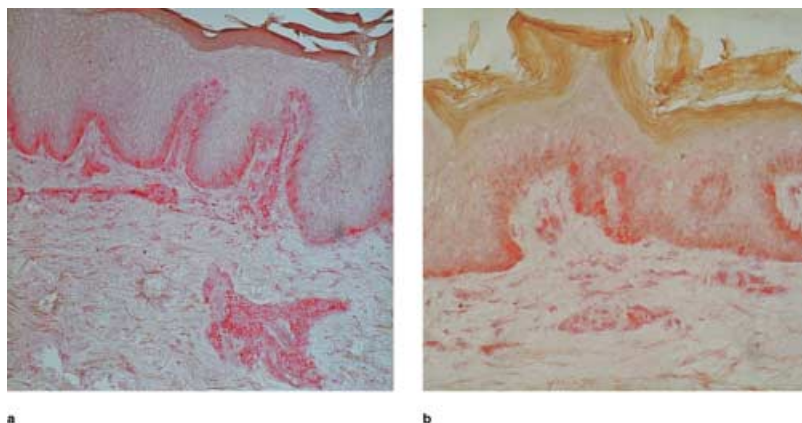


fig. 4 Immunohistochemical detection of anti-Bcl-2. *Left*, untreated keratinocytes along the basal layer exhibit positive staining with anti-Bcl-2; *right*, significant reduction in Bcl-2 expression in psoriatic epidermis after irradiation with 308-nm excimer light (original magnification $\times 100$).

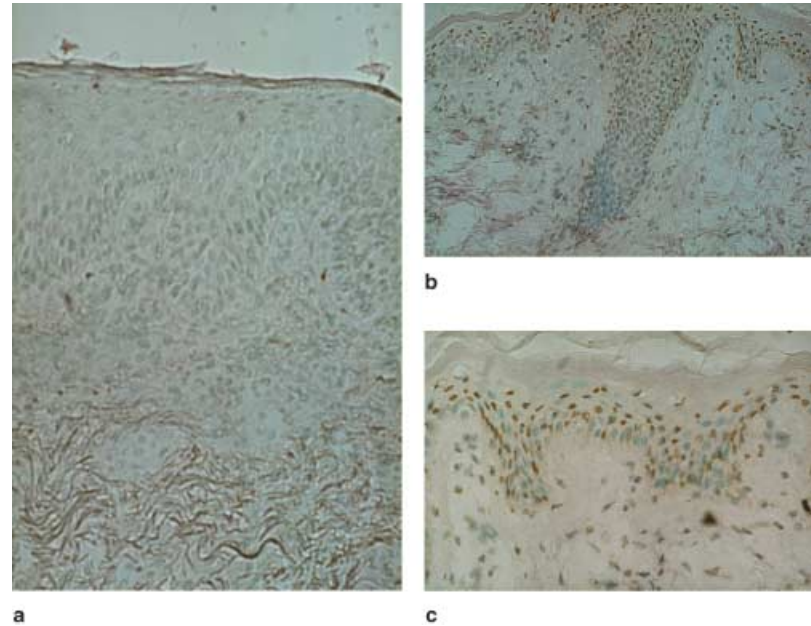


fig. 5 TUNEL-stained section of psoriatic untreated (a) and MEL-irradiated epidermis (b) (original magnification $\times 200$). (c) higher magnification highlighting the nuclear staining with TUNEL analysis in treated skin (original magnification $\times 400$).

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