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Keratinocyte dysfunction in vitiligo epidermis: cytokine microenvironment and correlation to keratinocyte apoptosis

Silvia Moretti[1], Paolo Fabbri[1], Gianna Baroni[2], Samantha Berti[1], Daniele Bani[3], Emilio Berti[4], Romina Nassini[5], Torello Lotti[1] and Daniela Massi[2]

[1]Department of Dermatological Sciences, University of Florence, Florence, Italy, [2]Department of Human Pathology and Oncology, University of Florence, Florence, Italy, [3]Department of Anatomy, Histology and Forensic Medicine (Histology Section), University of Florence, Florence, Italy, [4]Department of Medicine, Prevention and Biotechnology, University of Milan-Bicocca, Milan, Italy and [5]Department of Preclinical and Clinical Pharmacology, University of Florence, Florence, Italy

Summary. Vitiligo is a skin disorder characterized by loss of functional melanocytes. Keratinocytes contribute to melanocyte homeostasis, and keratinocyte alteration may play a role in melanocyte dysfunction in vitiligo. In particular, the release of melanogenic mediators and the level of functioning keratinocytes may affect melanocyte dysfunction in vitiligo epidermis. Keratinocyte-derived mediators involved in pigmentation, analysed by in situ hybridization, and epidermal apoptosis, detected by TUNEL assay and electron microscopy, were evaluated in lesional and perilesional skin biopsies from 15 patients with active vitiligo and in 5 control subjects. Among the melanogenic mediators, stem cell factor (SCF) and endothelin-1 (ET-1) mRNA were significantly reduced in lesional as compared to perilesional epidermis, whereas no difference was observed in mRNA of basic fibroblastic growth factor (bFGF) and granulocyte-monocyte colony stimulating factor (GM-CSF). The expression of mRNA for tumor necrosis factor (TNF)-α and interleukin-6 (IL-6), two pro-inflammatory cytokines with an inhibitory effect on pigmentation, was increased in the epidermis from vitiligo biopsies, whereas their expression was practically undetectable in the skin of control subjects. Apoptotic keratinocytes were more abundant in lesional vs. perilesional skin of vitiligo patients and were absent in the epidermis of control subjects. Changes in expression of keratinocyte-derived mediators observed in the present study are consistent with their differential functions in melanocyte regulation. In particular, increased TNF-α could contribute to keratinocyte apoptosis, which results in reduced release of melanogenic cytokines and ultimately in melanocyte disappearance.

Key words: Vitiligo, Apoptosis, Keratinocytes, Cytokines

Offprint requests to: Prof. Daniela Massi, Dipartimento di Patologia Umana ed Oncologia, Università degli Studi di Firenze, Viale G.B. Morgagni 85, 50134 Firenze, Italy. e-mail: daniela.massi@unifi.it

Introduction

Vitiligo is an acquired skin pigmentation disorder characterized by achromic patches due to loss of functional melanocytes. This condition affects 0.1-2% of the population and can be aesthetically disfiguring, especially in dark people (Hann and Nordlund, 2000). The precise mechanism which eventually results in melanocytes disappearance from the depigmented lesions remains unclear. Autoimmune, neural and metabolic hypotheses, which have been suggested as the underlying mechanisms of this disease (Moretti et al., 2006), are not completely satisfactory. In recent years, a complex melanogenic cytokine network between skin cells which regulate melanocyte activity by paracrine mechanisms has been demonstrated (Imokawa, 2004). In this regard, keratinocytes, the most abundant epidermal cell type, appear to play a major role. Keratinocytes surround pigmented cells and form a structural and functional unit with melanocytes, which is currently referred to as the epidermo-melanic unit (Jimbow, 1976). In this specific morphological arrangement, keratinocytes control melanocyte functioning and survival. Functional changes occurring in vitiligo skin have been related to structural abnormalities of keratinocytes (Moellmann et al., 1982; Bhawan and Bhutani, 1983; Maresca et al., 1997), most likely due to

oxidative stress of keratinocytes (Dell'Anna and Picardo, 2006).

Keratinocytes produce several mediators, cytokines and growth factors that support the growth and/or melanization of surrounding melanocytes (Imokawa, 2004). Among these molecules, endothelin (ET)-1 and stem cell factor (SCF) are intrinsic/constitutive mitogens and melanogens for human melanocytes and contribute to UVB-induced pigmentation in vivo (Imokawa et al., 1992; Grichnik et al., 1998; Imokawa, 2004). Granulocyte-monocyte colony-stimulating factor (GM-CSF) also plays an important role in pigment cell proliferation and UVA-induced pigmentation (Imokawa et al., 1998; Imokawa, 2004). Basic fibroblastic growth factor (bFGF) is a paracrine mitogen for human melanocytes (Halaban et al., 1988). Keratinocytes can secrete additional cytokines, such as IL-6 and tumor necrosis factor (TNF)-α, which function as paracrine inhibitors of melanocytes (Swope et al., 1991). Altered levels of keratinocyte-derived mediators have been recently described in vitiligo epidermis (Moretti et al., 2002a,b; Kitamura et al., 2004; Lee et al., 2005), suggesting a role of epidermal cytokines in the pathogenesis of vitiligo. Non conclusive and sometimes opposing results on the levels of these keratinocyte-derived mediators have been reported in vitiligo epidermis (Moretti et al., 2002a,b; Kitamura et al., 2004; Lee et al., 2005), most likely because of differences in methods and in patient selection. In addition, TNF-α, which has been found to be highly expressed in vitiligo skin (Moretti et al., 2002a,b; Grimes et al., 2004), is able to induce keratinocyte apoptosis in vitro (Reinartz et al., 1996) and in vivo (Meng et al., 1999), and keratinocytes in vitiligo lesions have been reported to be more susceptible to apoptosis (Lee et al., 2004, 2005).

In this study, we have investigated the expression of six keratinocyte-derived mediators with opposing roles in the proliferation and survival of melanocytes in the skin of vitiligo patients and healthy controls. In these skin tissue specimens the number of apoptotic cells was also measured. Results indicated that in vitiligo the expression of mitogenic mediators was decreased, whereas increased cytokines with inhibitory function were associated with augmented melanocyte apoptosis.

Material and methods

Tissue sampling

Fifteen patients affected by non segmental vitiligo in active phase and not submitted to local or systemic specific therapy in the previous four months were enrolled for the study. The study was approved by the local Ethical Committee and informed consent was obtained from each patient. Clinical features of patients are given in Table 1. Four-mm punch biopsies were taken from all patients in lesional and perilesional skin from new or enlarging lesions, in a period ranging from late autumn to early spring (to minimize the effects of sun exposure). Healthy skin from non-tanned, comparable sites of five healthy subjects was taken as control. Biopsies were fixed in buffered 10% formalin and embedded in paraffin. In three cases of vitiligo and three controls, a small part (1/4) of the biopsy was processed for transmission electron microscopy, as described below.

Immunohistochemistry (IHC)

Epidermal melanocytes were identified in tissue sections by anti-tyrosinase immunohistochemistry. Five-micron thick sections, 1 from each biopsy, were de-waxed in Bio-Clear (Bio-Optica, Milan, Italy) and re-hydrated in graded ethanol. Antigen retrieval was routinely performed by microwave pre-treatment (Microwave MicroMed T/T Mega, Milestone, Bologna, Italy) in EDTA buffer, pH 8.0 for 30 min. Then, the sections were placed in 3% H_2O_2 to block endogenous peroxidase. An anti-tyrosinase primary antibody (NeoMarkers, Fremont, CA) was applied at a 1:50 dilution for 1 h. at room temperature. Immune reaction was revealed with a streptavidin-peroxidase UltraVision Kit (Lab Vision, Fremont, CA) using diaminobenzidine (DAB) as chromogen. Nuclei were counterstained with Mayer's haematoxylin. A negative control sample was included in each run by omitting the primary antibody. Cutaneous melanoma was used as a positive control.

In situ hybridization (ISH)

Five-micron thick sections were obtained from each biopsy specimen and de-waxed with xylene, rehydrated in graded ethanol, rinsed in deionised water and treated with 20μg/ml proteinase K (Dako, Carpinteria, CA) in Tris-HCl, pH 7.5, at 37°C for 15 min. The sections were immersed in a pre-hybridization solution (50% formamide/4xSSC) to prevent background staining and

Table 1. Clinical details of patients.

Patient	Age (years)	Sex	Disease duration	Biopsy site
#1	75	F	12 years	Right thigh (anterior face)
#2	67	F	10 years	Left forearm (flexural surface)
#3	36	M	13 years	Left hand (dorsal surface)
#4	64	F	1 year	Abdomen
#5	37	F	4 months	Left foot (dorsal surface)
#6	55	F	20 years	Left hand (dorsal surface)
#7	28	M	2 years	Left shoulder
#8	51	F	18 years	Left arm (flexural surface)
#9	29	F	11 years	Right thigh (anterior face)
#10	42	M	6 years	Right hand (dorsal surface)
#11	29	F	3 years	Right elbow
#12	31	F	2 years	Left hand (dorsal surface)
#13	40	F	1 year	Left thigh (anterior face)
#14	52	M	5 years	Abdomen
#15	35	M	4 years	Right hand (dorsal surface)

then incubated with double FITC-human cytokine HybriProbe (Biognostik, Germany) overnight at 30°C. Probes of ET-1, SCF, bFGF, GM-CSF, TNF-α and IL-6 were employed (20 units for 1000 µl HybriBuffer ISH). Detection and visualization procedures were made with anti-FITC/AP monoclonal antibody (Vector Laboratories, CA) and BCIP/NBT as substrate. An additional amplification method was used for bFGF, GM-CSF and IL-6, using an anti-FITC/HRP polyclonal rabbit (Dako), followed by a Tyramide signal amplification system (Dako) and thereafter streptavidin-conjugated alkaline phosphatase (Dako) and BCIP/NBT as substrate. Sections were counterstained with neutral red. A negative control was performed by omitting the probe. Positive controls for each cytokine were: macrophages of lichen planus for GM-CSF; mononuclear infiltrate of lichen planus for IL-6; mast cells of mastocytoma for SCF; endothelial cells of scar neoangiogenesis for ET-1; keloid fibroblasts for bFGF; epidermis of scarring alopecia of discoid lupus for TNF-α. Reactive keratinocytes were scored as percentage of positive cells over total keratinocytes of the entire length of epidermis (under high-power fields). At least two sections for each staining were examined by 2 independent observers (SM and DM): discrepancies in the reading were resolved by a second reading of the slides until consensus was reached.

Terminal deoxynucleotidyl-transferase-mediated UTP end labelling (TUNEL) assay

This was carried out on 5-micron thick sections from each biopsy by a TUNEL detection kit (Apotag, HRP kit; DBA, Milan, Italy) according to the manufacturer's instructions. Briefly, sections were de-waxed, rehydrated and incubated with 15 mg/ml proteinase K for 15 min. at room temperature and then washed with PBS. Endogenous peroxidase was inactivated by 3% H_2O_2 for 5 min. at room temperature and then washed with PBS. Sections were immersed in terminal deoxynucleotidyltransferase (TdT) buffer containing deoxynucleotidyl transferase and biotinylated dUTP in TdT buffer, incubated in a humid atmosphere at 37° C for 90 min, and then washed with PBS. Finally, the sections were incubated at room temperature for 30 min with peroxidase-conjugated avidin and the signal was visualized with diaminobenzidine. The percentage of epidermal apoptotic cells over the whole cells in the epidermis was calculated on at least two sections for each sample by 2 independent observers, as above.

Electron microscopy

The tissue specimens were fixed in a mixture of 2% formaldehyde and 2.5% glutaraldehyde in 0.1 mmol/L cacodylate buffer, pH 7.4, at 5°C for 3 h., followed by post-fixation in 1% OsO_4 in phosphate buffer, pH 7.4 at room temperature for 2 h. The specimens were dehydrated in graded acetone, passed through propylene-oxide and embedded in Epon 812. Semithin sections were cut and stained with toluidine blue, while ultrathin sections were stained with uranyl acetate and alkaline bismuth sub-nitrate and examined under a JEM 1010 transmission electron microscope (Jeol, Tokyo, Japan) at 80kV.

Statistical analysis

Results are expressed as mean±SEM. Differences in mean values of cytokine expression and apoptotic cells in the epidermis between lesional versus perilesional skin were evaluated using the non-parametric Wilcoxon Rank test for paired samples. Differences between healthy control skin and perilesional and lesional vitiligo skin were assessed using the non-parametric Mann-Whitney U-test for independent samples. Differences were considered significant at $p<0.05$.

Results

Cytokine mRNA expression in the epidermis

The pattern of cytokine mRNA expression in the epidermis is summarized in Table 2. In the healthy control skin, the melanocyte-stimulating cytokines SCF and ET-1 showed the highest expression, followed by

Table 2. Cytokine expression in vitiligo and statistical analysis.

Cytokines	Mean ± SE [a]			P value [b]	P value [c]	P value [d]
	lesional (n=15)	perilesional (n=15)	healthy (n=5)			
SCF	53.7 ± 9.7	75.8 ± 5.7	100 (0)	0.038	0.003	0.003
ET1	50 ± 6.5	75.4 ± 5.5	100 (0)	0.005	0.001	0.001
bFGF	62,9 ± 9.6	54.6 ± 7.2	14 ± 6.5	NS	0.019	0.003
GMCSF	49.6 ± 8.7	42.5 ± 7.3	35 ± 12.2	NS	NS	NS
TNF-α	84.2 ± 5.6	75.4± 4.2	0	0.042	0.001	0.001
IL6	26.5 ± 4	36.2 ± 6.1	0	NS	0.001	0.001

NS: not significant ($p>0.05$); [a]: Cytokines have been measured and presented as percentage of epidermis that was judged to have reacted (as mean ± ES); [b]: Lesional vs. perilesional (Wilcoxon test for paired samples); [c]: Lesional vs. healthy (Mann-Whitney test for independent samples); [d]: Perilesional vs. healthy (Mann-Whitney test for independent samples).

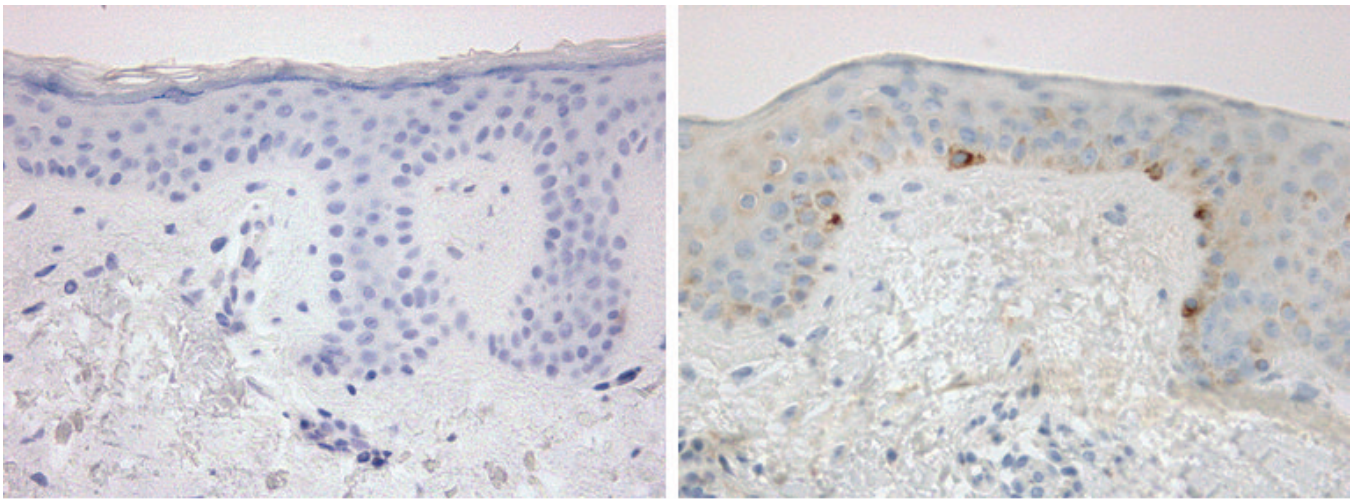

Fig. 1. Immunostaining for tyrosinase in lesional **(a)** and perilesional **(b)** vitiligo epidermis. x 200

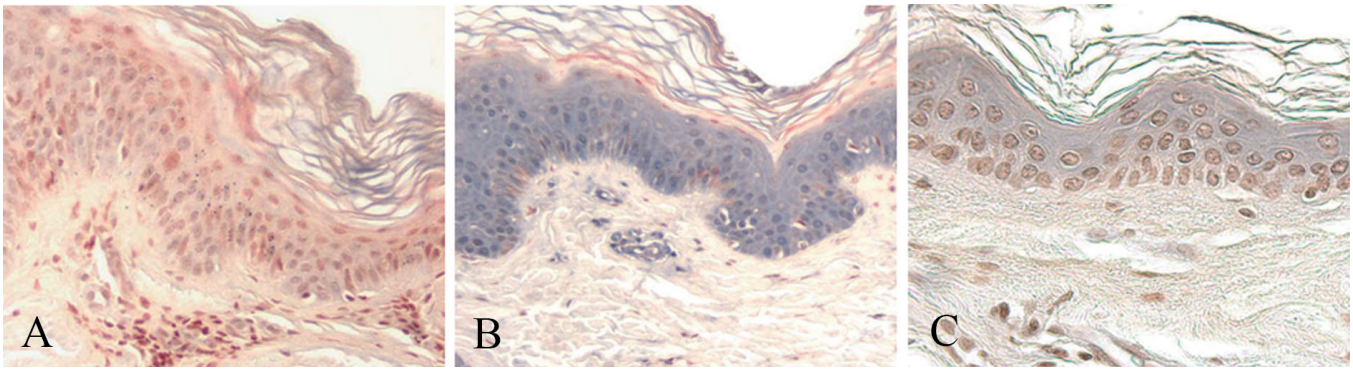

Fig. 2. Expression of SCF transcript in lesional **(a)** and perilesional **(b)** vitiligo epidermis and in healthy control skin **(c)** by in situ hybridization. Original magnification, x 20

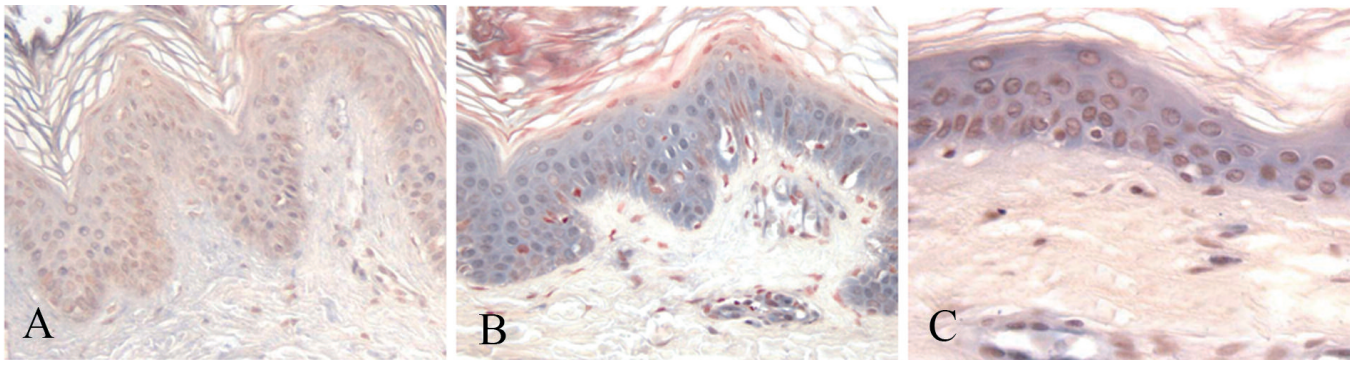

Fig. 3. Expression of ET-1 transcript in lesional **(a)** and perilesional **(b)** vitiligo epidermis and in healthy control skin **(c)** by in situ hybridization. Original magnification, x 20

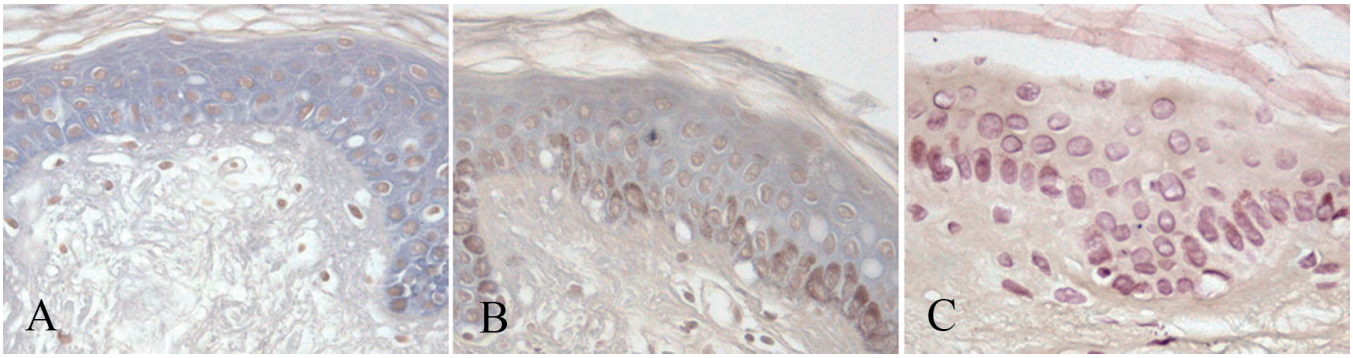

Fig. 4. Expression of TNF-α transcript in lesional **(a)** and perilesional **(b)** vitiligo epidermis and in healthy control skin **(c)** by in situ hybridization. Original magnification, x 20

bFGF and GM-CSF, which exhibited a lower staining. Pro-inflammatory, melanocyte-inhibiting cytokines IL-6 and TNF-α were not detected. In the lesional skin from vitiligo patients, the expression of SCF and ET-1 was significantly lower than in the skin from healthy control as well as, although to a lesser extent, in perilesional biopsies of vitiligo patients. Expression of the mRNA of bFGF was significantly higher in both lesional and perilesional skin versus the skin of healthy controls, whereas no significant difference was observed between lesional and perilesional skin. Although there was a tendency to a lower expression in the normal healthy skin, GM-CSF showed no significant changes between the three groups analyzed. TNF-α mRNA was highly expressed in both lesional and perilesional skin, with a significantly higher reactivity in achromic lesional skin than in the skin of healthy controls. IL-6 mRNA values in vitiligo biopsies were significantly higher than in the skin of healthy controls, with no appreciable differences between lesional and perilesional skin. Lesional vitiligo samples could be easily recognized from perilesional samples due to the complete absence of melanin, as well as tyrosinase-positive melanocytes in the epidermis. Representative images of epidermal reactivity for tyrosinase and of positive cells for cytokine transcripts by in situ hybridization are shown in Figures 1-4.

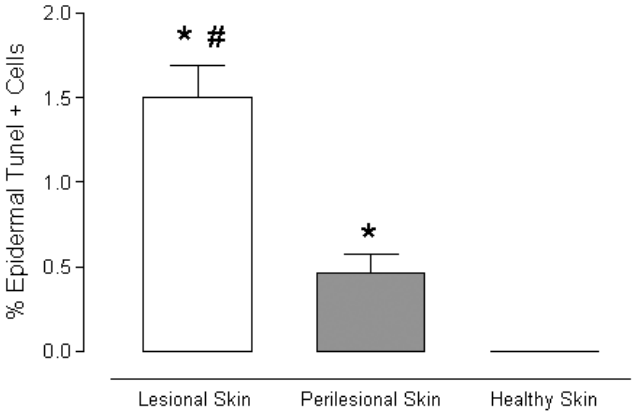

Fig. 5. Bar graph showing the mean percentage of apoptotic cells ± SEM in the epidermis of lesional (n=15) and perilesional vitiligo skin (n=15) and of healthy control skin (n=5). * P<0.05 vs. Healthy Skin.

Epidermal cell apoptosis

The results of the TUNEL assay, which detects epidermal cell apoptosis, are reported in Figure 5. No epidermal apoptotic cells were observed in any skin biopsies from the 5 healthy controls. In contrast, some scattered epidermal TUNEL- positive cells were observed in the lesional (1.5±0.2, p<0.05 vs. healthy skin) and perilesional skin (0.46±0.1, P<0.05 vs. healthy skin) of the 15 vitiligo patients. The number of TUNEL-positive cells was also significantly higher in the lesional epidermis vs. the perilesional skin (P<0.05) of vitiligo patients. Apoptotic cells were mainly located in the lower epidermal layers (Fig. 6). TUNEL-positive cells were found in lesional vitiligo skin where no tyrosinase-positive melanocytes were detectable. Thus, they were identified as apoptotic keratinocytes. This assumption was supported by the ultrastructural analysis (see below).

Ultrastructural analysis for cell signs of apoptosis

Ultrastructural examination of the epidermis from the perilesional and lesional skin of 3 vitiligo patients showed that, in the perilesional tissue, keratinocytes usually contained numerous melanosome-filled vacuoles in their cytoplasms, especially in the basal and juxtabasal layers. Infiltrating inflammatory cells or signs of keratinocyte apoptosis were not observed. On the other hand, in the lesional epidermis, keratinocytes were devoid of melanosome-containing vacuoles and some of them showed clear-cut signs of early apoptosis, such as clearing of the cytoplasmic matrix and swollen mitochondria with disruption of cristae (Fig. 7).

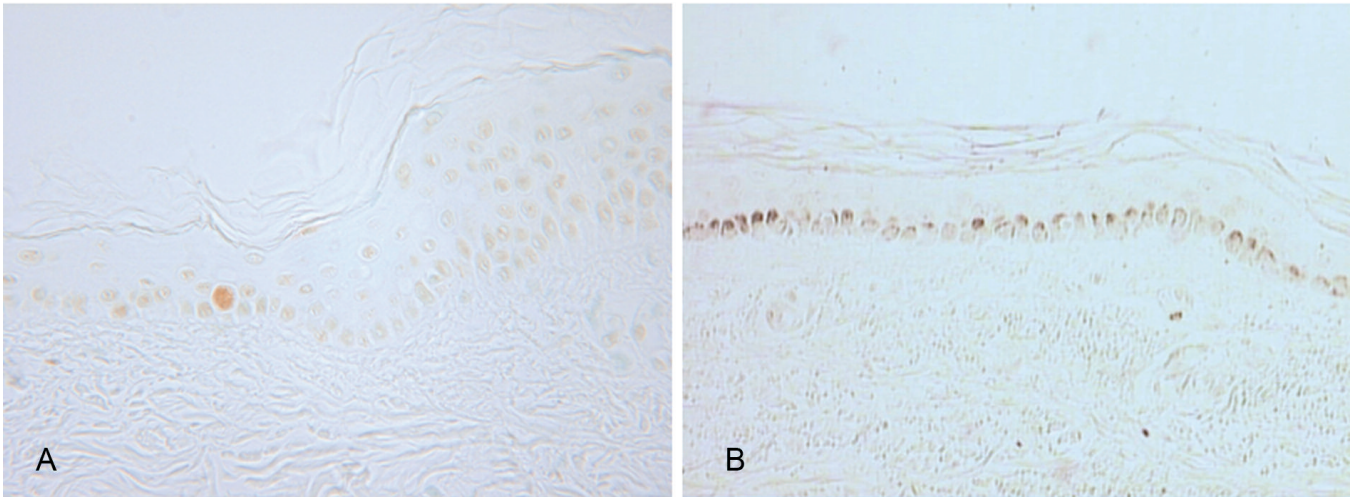

Fig. 6. TUNEL assay for apoptosis. An apoptotic basal keratinocyte (with no melanin granules) is detected in lesional vitiligo epidermis **(a)**; no TUNEL-positive cells can be seen in normal healthy epidermis **(b)**. x 300

Scattered vacuolar degeneration in basal and supra-basal epidermal layers and extracellular granular material reminiscent of apoptotic bodies were seldom observed (data not shown). As expected, no melanocytes were detected in the lesional epidermis.

Discussion

Keratinocytes of the epidermo-melanic unit exert a complex regulatory influence on melanocytes. In vitiligo, keratinocytes show morphological and functional abnormalities (Moellmann et al., 1982; Bhawan and Bhutani, 1983) which have been considered to affect melanocyte growth and survival in vitro (Birol et al., 2006). Although reported results have not always been consistent, an abnormal production of keratinocyte-derived cytokines with paracrine effects on melanocyte function has been recently demonstrated in vitiligo epidermis (Moretti et al., 2002a,b, Grimes et al., 2004; Kitamura et al., 2004; Lee et al.,2005), and a higher susceptibility to apoptosis of keratinocytes has been described in vitiligo (Lee et al., 2004, 2005). Thus, an impaired keratinocyte function may represent an underlying mechanism which contributes to melanocyte damage in vitiligo. Nevertheless, it is noteworthy that both the inflammatory melanocyte-inhibiting cytokines resulted significantly higher in vitiligo epidermis than in normal control skin, stressing a remarkable difference between pathologic and normal cutaneous conditions.

In the present study we identified a specific mRNA expression profile of melanocyte-stimulating mediators. If lower levels of transcripts for some melanogenic mediators, ET-1 and SCF, were observed, no significant difference was found for others, such as bFGF and GM-CSF in lesional versus perilesional skin. Regarding melanocyte-inhibiting cytokines, the levels of transcript for TNF-α were more elevated, while those for IL-6 were similar in lesional versus perilesional skin. However, both inflammatory cytokines were significantly higher in vitiligo than in healthy skin. Comparing vitiligo skin with normal skin, transcripts of SCF and ET-1 appeared to be significantly decreased, while transcript of GM-CSF showed a slight, but not significant, increase, and transcript of bFGF exhibited a significantly higher expression in vitiligo specimens. Thus, while the mRNA levels of SCF, ET-1, TNF-α and IL-6 matched the protein values detected by immunohistochemistry in our previous study, the mRNA levels of GM-CSF and bFGF appeared to be in disagreement with the protein amounts that were decreased in vitiligo compared to healthy skin (Moretti 2002a,b). Such a discrepancy between mRNA and protein expression is not so infrequent, since pioneer studies by Anderson and Seilhamer demonstrated that there is not always a good correlation between transcript and protein levels (Anderson and Seilhamer, 1997). In our case, a post-transcriptional down-regulation or a lower protein stabilization could be taken into account in vitiligo skin to explain this disagreement.

We believe that in situ hybridization, with accurate tissue sampling to preserve mRNA integrity, is a more sensitive and specific assay than immunohistochemistry. However, due to the limited number of cases, it is also possible that the divergent results derive from the different group of patients analyzed.

We found that the expression of intrinsic paracrine mediators involved in epidermal hyper-pigmentation, SCF and ET-1 (Imokawa, 2004), were significantly reduced in the lesional vitiligo skin vs. both control skin and perilesional vitiligo skin. This finding further supports the hypothesis that these mediators contribute to melanocyte survival (Moretti et al., 2002a,b, Lee et

al., 2005). bFGF has not yet received a precise role as a constitutive mitogen or melanogen inductor for human melanocytes, because it requires co-factors for the melanocyte stimulation (Imokawa, 2004). Accordingly, its role in vitiligo remains unclear. Present findings, with a higher expression of bFGF in the epidermis of vitiligo patients compared to the healthy controls, could be ascribed to a compensatory mechanism aimed to balance the reduction of intrinsic/constitutive melanogen factors. The observation that GM-CSF expression is similar in vitiligo and normal skin suggests a negligible role for this mediator (Imokawa, 2004).

The expression of both TNF-α and IL-6 transcripts was nearly undetectable in the biopsies from healthy subjects, whereas it progressively increased in perilesional and lesional vitiligo biopsies, suggesting that the epidermis of vitiligo patients produces these pro-inflammatory and lymphocyte recruiting (Dell'Anna and Picardo, 2006) cytokines, which may inhibit melanocytes. Recruited CD8+ cytotoxic lymphocytes

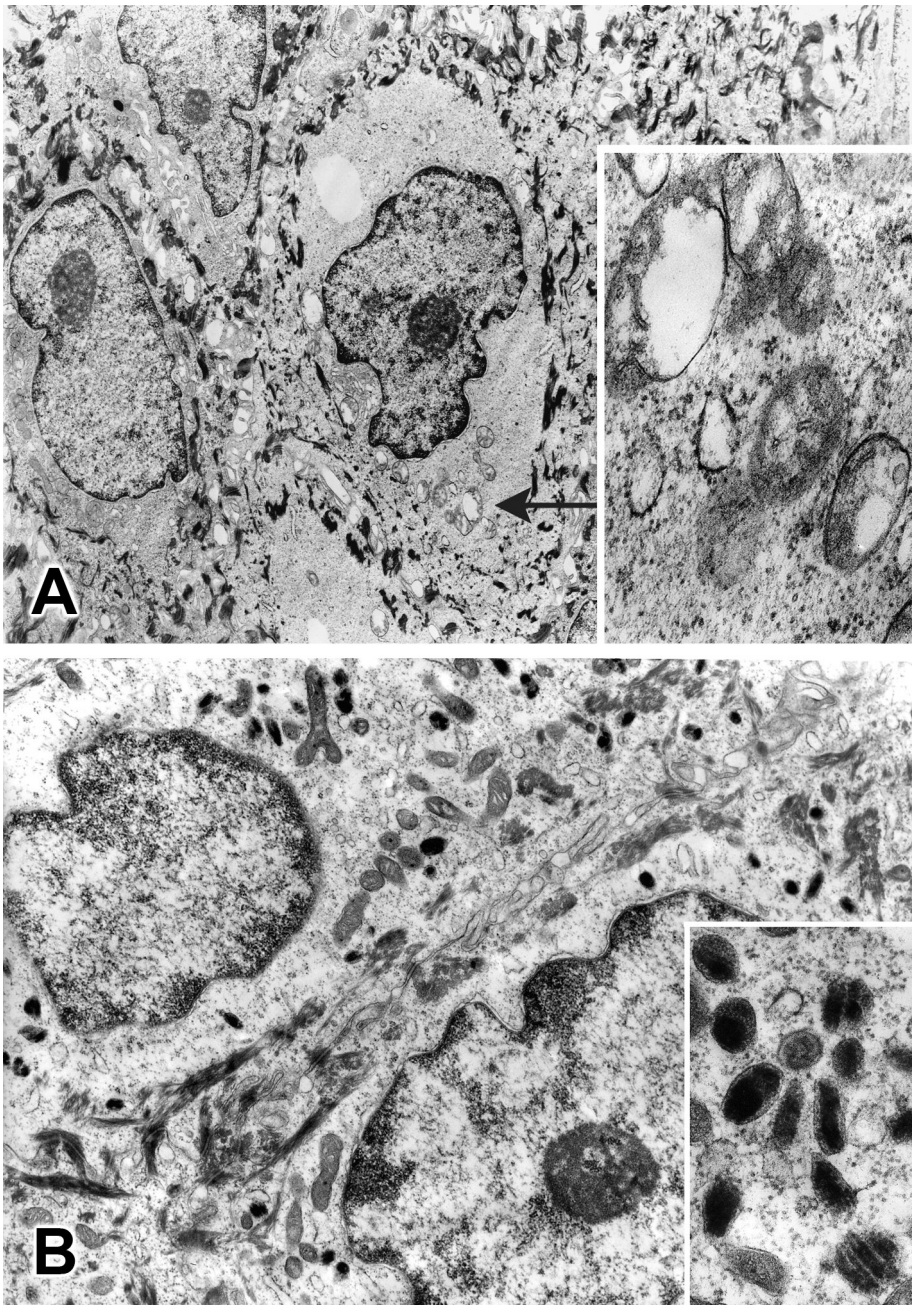

Fig. 7. Representative electron micrographs from vitiligo biopsies. **A.** A keratinocyte from lesional vitiligo shows signs of early apoptosis, i.e., clearing of the cytoplasmic matrix and swollen mitochondria. x 10,000. **Right inset:** a detail of the mitochondria, with disruption of cristae and clearing of the inner matrix. x 62,000. **B.** Keratinocytes from perilesional vitiligo show numerous melanosomes in their cytoplasms. x 20,000. **Right inset:** a detail of the melanosomes. x 50,000

specifically directed toward melanocyte melanosomal antigens may result in melanocyte loss from vitiligo epidermis, in agreement with the autoimmune pathogenesis of vitiligo (Ongenae et al., 2003) and also in agreement with a similar pathomechanism described in hypopigmented mycosis fungoides (Singh et al., 2006). The increase in TNF-α in vitiligo skin is consistent with previous findings, which showed in vitiligo skin an increase in protein expression (Moretti et al., 2002a,b; Birol et al., 2006; Kim et al., 2007) or a reduction in TNF-α transcript expression after topical treatment with the immuno-modulatory drug tacrolimus (Grimes et al., 2004). Thus, a pathogenic involvement of this cytokine in vitiligo is further supported, although other important pathomechanisms are surely involved, because a TNF-α inhibitor mono-administration in a small series of vitiligo patients did not show efficacious therapeutic response (Rigopoulos et. al. 2007).

The novel finding of the present paper is derived from the simultaneous observation of increased TNF-· levels and the occurrence of apoptotic keratinocytes in vitiligo skin biopsies observed by TUNEL assay and confirmed by electron microscopy. This cytokine may induce keratinocyte apoptosis in vitro (Reinartz et al., 1996) and in vivo (Meng et al., 1999). These results appear to be in agreement with a recent paper reporting that vitiliginous keratinocytes are more susceptible to TNF-α mediated apoptosis through an impaired activation of downstream signaling molecules (Kim et al., 2007).

In our study, keratinocyte apoptosis was associated with reduced SCF levels in the achromic versus the pigmented epidermis, a hypothesis that can also be supported by the present results. Our data are consistent with the report of increased keratinocyte apoptosis in depigmented suction-blistered epidermis compared to its pigmented counterpart described in vitiligo patients, associated with a lower expression of SCF in achromic vitiligo patches (Lee et al., 2005). In addition, deprivation of SCF in the culture medium of a keratinocyte feeder layer was found to reduce the amount of co-cultured melanocytes (Lee et al., 2005), and the in vitro proliferation rate of melanocytes was enhanced by the conditioned medium of tacrolimus-treated keratinocyte cultures, in which SCF concentration increased dose-dependently upon tacrolimus treatment (Lan et al., 2005). All the above findings underscore the pivotal role of keratinocyte-derived SCF and TNF-α in regulating melanocyte growth and functional activity. An alternative hypothesis is that, in vitiligo skin, increased TNF-α levels induce keratinocyte apoptosis (Reinartz et al., 1996; Meng et al., 1999), thus impairing keratinocyte activity and blunting secretion of paracrine melanogenic cytokines, such as SCF (Imokawa, 2004) and indirectly causing melanocyte dysfunction and demise (Lee et al., 2005). Down-regulation of the SCF specific receptor c-Kit and its MITF transcription factor by melanocytes in vitiligo epidermis (Kitamura et al., 2004) has also been reported, further supporting the view that the SCF/c-Kit system is involved in melanocyte dysfunction in vitiligo.

In conclusion, here we have shown that the epidermal mediators and cytokine microenvironment in lesional and perilesional vitiligo differs from that of healthy control skin. This difference consists of decreased expression of SCF and ET-1, two melanocyte-stimulating mediators, and increased expression of TNF-α and IL-6, well known pro-inflammatory cytokines, with melanocyte-inhibiting functions. We also found that keratinocyte apoptosis is associated with vitiligo skin. Thus, a peculiar change in mediator level in the vitiligo milieu may contribute to keratinocyte dysfunction and apoptosis, exaggerating a vicious cycle that eventually results in the disappearance of functional melanocytes.

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