tion regimes using a highly tumorigenic and poorly immunogenic melanoma cell line have demonstrated that tumor regression and subsequent depigmentation show an absolute requirement for $CD8^+$ cells (16). Other studies support this hypothesis and as such many new therapeutics aim to augment the action of cytotoxic $CD8^+$ T cells (17,18).

However, the current focus on $CD8^+$ T cells may be deflecting attention from the equally or more important role of $CD4^+$ T cells in initiating melanocyte-specific autoimmunity (13,19,20). Clonotype-specific $CD4^+$ T cells have been more difficult to identify by tetramer staining, but recent findings suggest that these cells are of critical importance in mediating cutaneous autoimmunity. In addition to sustaining and regulating the humoral and cellular responses, $CD4^+$ T can also act in the absence of B cells and $CD8^+$ T cells to selectively target melanocytes for cell death, an action which is partially dependent on Fas–FasL signalling (20). The immunosuppressive role of antigen-specific $CD4^+$ Treg cells in dampening autoimmune responses also needs to be considered in the design of effective treatments for both melanoma and vitiligo (21,22). Thus, $CD4^+$ T cells play a multi-faceted role in cutaneous disease and moving $CD4^+$ T cells to centre stage may help to increase the range of therapeutic options in these prevalent conditions.

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Viewpoint 5

Most ongoing research on vitiligo is based on the long-standing hypothesis that in vitiligo lesions melanocytes are actually lost rather than inactivated. Before entering into the details of vitiligo pathogenesis, it is worth revisiting briefly the evidence that supports this hypothesis.

Loss of epidermal melanocytes in vitiligo: any reason to doubt?

Most studies based on standard histology, electron microscopy and immunohistochemistry suggest that melanocytes are absent from vitiligo lesions (1). However, if vitiliginous melanocytes were inactivated, one might not expect melanocyte-specific antigen expression at the protein level in these cells. Two studies have reported findings that are compatible with this view. First, melanocytes cultures were successfully established from depigmented epidermal suction blister tissue of 12 patients with vitiligo (2). Secondly, tyrosinase and DCT mRNA could be detected in vitiliginous skin of three patients by RT-PCR (3).

Although scarce, this type of findings should not be overlooked, and it may be worth searching for additional melanocyte-specific transcripts in vitiliginous skin. In addition, an analysis of repigmentation patterns in patients treated for vitiligo shows that while PUVA therapy induces perifollicular repigmentation, steroids induce diffuse repigmentation. This suggests that, at least in some vitiligo lesions, follicular melanocytes might not be the unique source of melanin production during repigmentation (4). It would be interesting to gain more insight into repigmenting vitiligo lesions by means of modern skin imaging techniques such as cutaneous spectrophotometry (Siascopy) or *in vivo* confocal microscopy.

Persistance of follicular melanocytes in vitiligo: why are they spared?

While the absence of epidermal melanocytes in vitiligo has been the focus of numerous studies, the sparing of follicular melanocytes has been less investigated. Developmental differences can account for the different fates of epidermal and follicular melanocytes, as lower portions of mouse hair follicles are known to contain stem cells of the melanocyte lineage (5). In the case of hair greying caused by defective

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self-maintenance of melanocyte stem cells, the process is accelerated dramatically with Bcl2 deficiency, which causes selective apoptosis of melanocyte stem cells, but not of differentiated melanocytes (6).

The pale ear (ep) mouse strain is a model for the Hermansky–Pudlak syndrome type 1 (HPS-1), an autosomal recessive disorder causing pigmentary dilution. The ep mice have a normal coat colour but their ears and tails are hypopigmented. In a recent study, it was determined that in the tail of ep mice, the defective gene causes delayed onset of interfollicular epidermal melanocyte tyrosinase activity, decreased numbers of interfollicular melanocytes, and severe immaturity of tail epidermal melanosomes, findings not observed in follicular melanocytes. These results are intriguing in light of vitiligo – a disease that affects interfollicular and follicular melanocytes differentially. It is interesting to speculate that the gene products of ep could be the targets for the autoimmune process (7).

Apoptotic mechanisms in normal melanocytes: how are they involved in vitiligo?

There are at least two well-defined pathways to cell death: necrosis (resulting from extrinsic cytotoxic factors) and apoptosis (a carefully regulated intrinsic cell death programme in which two sets of pro-apoptotic and anti-apoptotic molecules antagonize and balance each other).

Normal human melanocytes express high levels of anti-apoptotic proteins (Bcl2 and Bcl-XL) and of the pro-apoptotic protein Bax, with emphasis on the Bcl2/Bax ratio (8). In vitiligo, histology and electron microscopy support the occurrence of melanocyte apoptosis. Ultrastructurally, melanocytes exhibit anomalies such as nuclear shrinkage, vacuolization, loss of dendrites and detachment (9). TNF-related apoptosis-inducing ligand (TRAIL) promotes very efficiently apoptosis of primary human melanocytes through activation of caspases. Stem cell factor (SCF) strongly protects melanocytes from TRAIL-induced apoptosis, through the activation of the phosphatidyl-inositol-3-kinase (PI3K) pathway and its downstream target AKT (10). The status of the PI3K/AKT pathway in vitiligo remains to be investigated.

Stem cell factor induces its biological effects via the tyrosine kinase receptor c-KIT which is expressed on melanocytes. The SCF/c-KIT pathway plays a critical role in the control of normal human melanocyte homeostasis (11). The binding of the ligand SCF to the c-KIT receptor triggers the activation of the MAP kinase pathway (12). In response to this activation, the melanocyte-specific, microphtalmia-associated transcription factor MITF-M is phosphorylated and activates the transcription of the anti-apoptotic gene Bcl-2 (13).

Interestingly, in vitiligo skin, a significantly lower expression of SCF was detected compared with perilesional, non-lesional and healthy skin (14). A recent study also demonstrated a lower expression of SCF in vitiligo keratinocytes (15). In addition, in perilesional skin of some patients with vitiligo, there is a reduction in the numbers of melanocytes expressing the c-KIT receptor (16). Finally, a downregulated expression of c-KIT and MITF-M was found at the edge of the lesional epidermis (17). In this context, there have been reports of vitiligo-like lesions in a significant percentage of patients treated with the SCF–c-KIT pathway inhibitor imatinib mesylate (18).

All these observations strongly suggest that dysfunction of the SCF/c-KIT/MITF-M signalling pathway in keratinocytes (SCF) and melanocytes (c-KIT and MITF-M) may be responsible for the survival defect and loss of melanocytes in vitiligo. Hepatocyte growth factor (HGF), a keratinocyte-derived factor, binds to the HGF receptor, a plasma membrane tyrosine kinase receptor on melanocytes. The Met proto-oncogene encodes for the HGF receptor. A recent report demonstrated that the cAMP pathway regulates Met through MITF that binds and activated the Met promoter. Furthermore, upregulation of Met by cAMP renders melanocytes responsive to the protective effects of HGF against apoptosis (19). The role of HGF and its receptor in the pathogenesis of vitiligo has not been evaluated yet.

Melanocyte environment in vitiligo: what about fibroblasts?

The molecular dissection of SCF/c-KIT or HGF/Met pathways has drawn attention to the keratinocyte–melanocyte interplay and its potential role in vitiligo. However, recent data suggest that fibroblasts may also deserve consideration. An analysis of gene expression in human fibroblasts derived from the skin at different anatomical sites showed that fibroblasts from each site displayed distinct and characteristic transcriptional patterns (20). Large-scale differences in the gene expression programmes were related to three anatomic divisions: anterior–posterior (rostral–caudal), proximal–distal (21). In line with these findings, it was shown recently that palmoplantar fibroblasts significantly suppressed the growth and pigmentation of melanocytes compared with non-palmoplantar fibroblasts. Fibroblasts derived from palmoplantar skin expressed high levels of dickkopf 1 (DKK1), and DKK1 decreased melanocyte function, probably through β-catenin-mediated regulation of microphthalmia-associated transcription factor activity (22,23). The results of this study are intriguing in light of vitiligo in which melanocyte loss demonstrates unique anatomic predilection (24).

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Identification of the first vitiligo-predisposing gene: time for innate immunity?

While autoimmunity is implicated in the pathogenesis of vitiligo, all studies including the most recent have underlined the role of melanocyte antigen-specific immunity (25–29). Recently, a large association mapping genetic study showed an association of specific variants of NALP1 with vitiligo alone, with an extended autoimmune and inflammatory disease phenotype, or with both (30). NALP1 encodes a regulator of the innate immune system. NALP1 is a direct sensor of bacterial components in host defense against pathogens. NALP1 responds to bacterial ligand muramyl-dipeptide (MDP). NALP1 participates in a multiprotein complex called inflammasome that activates caspase-1. Caspase-1 proteolysis activates IL-1β maturation (31). Anti-apoptotic proteins Bcl-2 and Bcl-XL bind and suppress NALP1, reducing caspase-1 activation and IL-1β production (32).

The next step will be to determine if innate immunity, caspase-1 and IL-1β are linked to melanocyte loss or if NALP1 exerts its role in vitiligo through a different pathway.

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