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## Viewpoint 2

The rise and fall of vitiligo involves an understanding of virtually every aspect of the melanocyte. When studying vitiligo aetiology, one comes across cell biology, genetics, biochemistry and immunology, even microbiology. The genetic predisposition to vitiligo is almost a given. In certain communities, the disease frequency can run up to more than 20% and for most patients, it is completely clear that vitiligo ‘runs in the family’, with more than 25% of patients assigning their disease to hereditary factors (1,2).

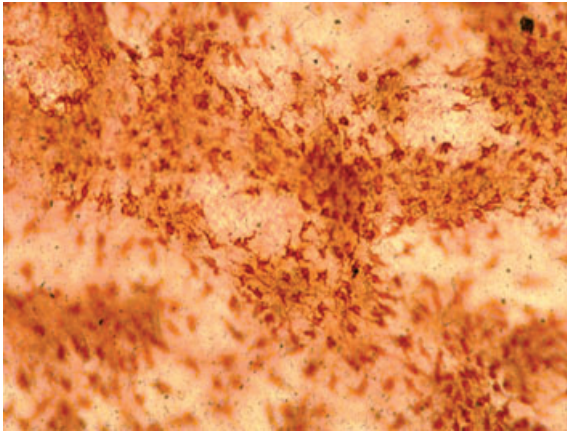
With major studies underway to identify genes unequivocally associated with vitiligo, the next step is to understand the biology of depigmentation. First and foremost, it was important to establish that melanocytes are lost from vitiligo lesions as the disease progresses, and that depigmentation is not merely a process whereby melanogenesis is inhibited (3). Secondly, an understanding of the interaction between melanocytes and other epithelial cells comes into play. There is the possibility that melanosome transfer to neighboring keratinocytes is inhibited, which should definitely impact on skin colour (4). There is also an as of yet incompletely understood intimate relationship between melanocytes and Langerhans cells, where one can almost disguise as the other in appearance and function (Fig. 1).

Knowing melanocytes as an immunologic entity within the skin has helped us to understand that melanocytes are ideal targets for an autoimmune response. After all, melanocytes produce cytokines that draw attention to themselves (5,6). They can phagocytize, and can process and

present antigens in the context of MHC class II as one would normally expect a professional antigen-presenting cell to do (7,8). These are not routine tasks, as we see melanocytes express HLA-DR specifically in vitiligo, and in melanoma (9–11). But is that not exactly when it is time to intervene? Exactly when melanocytes are involved in the disease with no easy cure in sight?

The properties of melanocytes that make them legitimate members of the skin immune system are often lost from the general sphere of attention as the focus is placed on the incredible task of melanization. Of course, their responsibility for skin colour puts melanocytes in the limelight, and helps protect us from harmful effects of UV irradiation (12,13). It also defines our ethnic heritage and is thereby a visible part of our identity (14). And many factors identified as precipitating factors in vitiligo, play into the biochemical process called melanogenesis, including overexposure to UV by stimulating melanization (15). Melanization is not so safe a process for melanocytes to begin with, unless it is contained with flawlessly formed melanosomes and none of those toxic intermediates ever comes in touch with the cells’ cytoplasm (16).

Other obvious precipitating factors are those known to cause ‘occupational vitiligo’, which can be oversimplified as a process of competitive inhibition with melanin precursors that melanogenic enzymes can convert into toxins within melanocytes only (17). Or even wounding or bruising, possibly involving inflammation followed by postinflammatory consequences that touch on melanization (18).



**Figure 1.** Melanocytes detected in split skin preparation. A forekin sample was salt split with NaBr and separated into epidermis and dermis. The epidermis was overturned and stained with antibody NKI-Beteb to human gp100 and detected in an indirect, peroxidase immunostaining with amino ethyl carbazole (AEC) as a substrate. Note the morphology and distribution pattern of melanocytes, which is very similar to a similar preparation stained for Langerhans cell markers.

The last word has not been said on the topic of antigen mimicry, where an infectious agent may carry antigens with homology to antigenic compounds otherwise found in melanocytes (19,20). Think of stress proteins, particularly immunogenic molecules that are very well conserved throughout evolution (21). These may bring a new twist to the current understanding of vitiligo aetiology and the interpretation of antigen mimicry, as stress proteins can chaperone proteins specific to the cells from which they are derived.

Taken together with the fact that melanocytes are phagocytic and are located within the barrier organ named skin, melanocytes are exposed to conditions and particles other cell types will never be in touch with. This is of particular importance as melanocytes are not famous for their mobility, and must stay put even if circumstances are particularly dangerous (22). Finally, melanocytes are not easily replenished and require several growth stimulants for proliferation (23). Taking all these factors into account, the autoimmune response that takes place within vitiligo skin may seem to be an epiphenomenon and something that does not need to be reckoned with.

Cellular infiltrates in vitiligo skin tend to be minute, involving just a few macrophages, dendritic cells and T cells plus perhaps some as of yet unidentified players, but in most cases the infiltrates are not observed at all (24,25). Infiltrating, melanocyte-reactive cytotoxic CD8<sup>+</sup> T cells may be more abundant in vitiligo skin but only scarcely is depigmentation accompanied by visible inflammation (26). Moreover, infiltrates are only found in a circumscribed region of rapidly progressing vitiligo, at the leading edge of depigmenting skin. So why bother with this aspect of vitiligo?

First of all, the progression of a lesion is most logically associated with an immune response. Why else will depigmentation progress beyond the region of skin that was exposed to any precipitating factor? Autoimmune reactivity defines the progressive nature of vitiligo and thereby the very aspect whereby it can be distinguished from other pigmentary disorders. But, more importantly, the autoimmune response offers a unique and essential point of intervention.

Short of fixing defective genes or replacing the gene products, treatment for vitiligo comes in two steps (27). Whereas transplantation offers a means of repigmentation once depigmentation has come to a halt, any successful therapeutic measure should involve (a) intervention with ongoing autoimmune reactivity; and (b) a repigmentation strategy. Careful UV exposure has aspects of both; transplantation measures can serve for (b) yet there is a wide array of immune interventions available.

What it will take to attract attention from pharmaceutical industry and to initiate clinical trials, even with existing biologicals is to officially recognize vitiligo as an autoimmune disorder. The evidence is close at hand. The body of work published on melanoma vaccine development has highlighted the problem of vaccine-associated autoimmune responses as patients with melanoma presented with progressive skin depigmentation in response to vaccines.

It has become clear that vitiligo is a positive prognostic factor in melanoma, and an understanding of antigens recognized by T cells and antibodies, factors that drive extravasation to the skin. The development of a regulatory response, introduction of immunosuppressive cytokines and/or factors that deplete any factor that forms a bottleneck in the development autoimmune vitiligo are all processes that can be exploited as the autoimmune response in vitiligo is better understood. Vitiligo deserves recognition as an autoimmune disease of the skin.

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### Viewpoint 3

Vitiligo has, for a long time, lacked a clear classification, and even the origin of the name is controversial, indicating that many aspects of vitiligo are still unclear. The definition of the disease reaches back to the dawn of the last century (1). A step forward was taken with the consensus provided by the Vitiligo European Task Force (2). Several theories have been proposed to explain the disappearance of functional melanocytes, when even the concept of 'disappearance' is a matter of debate (3–5). Several morphological, functional or metabolic alterations, apparently unrelated, have been described. One of the problems is that researchers work separately and have rarely tried to reproduce the data published by others and to combine these with their own results.

*In vitro* studies demonstrated intrinsic cellular damage, providing a clear indication for the involvement of apparently healthy melanocytes. Metabolic damage, characterized by increased susceptibility to chemical/physical stress, high H<sub>2</sub>O<sub>2</sub> intracellular level, low catalase activity, faulty activity of the mitochondrial respiratory chain and defective assembly/activity of TRP1, all have been demonstrated (6–11). The increased cellular death cannot be a simple effect of the *in vitro* manipulation, but mirror the *in vivo* experience of the Koebner phenomenon.

For us the main questions are: Is vitiligo due to a specific defect of the melanocytes? How do melanocytes disappear? Are the other epidermal cells affected? Is systemic involvement possible?

Current publications underline two facts: (a) even non-epidermal cells are affected; and (b) localized or even systemic oxidative stress occurs (12–14).

If the oxidative stress participates in vitiligo pathogenesis, further questions remain open: where does oxidative stress arise from? Is it generated by a unique cause? How could it be related to the large variety of observed alterations, such as pro-inflammatory cytokines and the production of keratinocyte growth factors (15–17)?

If the epidermal reactive oxygen species (ROS) hyperproduction were only due to the oxygen burst of the infiltrating polymorphonuclear cells (PMN) or to the excessive release of TNF- $\alpha$ , this is inconsistent with ROS overproduction even *in vitro*, where both PMN and TNF- $\alpha$  are absent (16,18).

An effort has to be made to identify a potential unifying process that can account for the described cellular impairments. The genetic approach suggests a wide range of potential, defective checkpoints, but the differences between the studied populations make it difficult to interpret the data (19–22). The genetic analyses indicate a possible pathogenic pathway mainly in those patients with vitiligo (25–40%) with an apparent auto-immune contribution. The description of the 'inflammosome' (23,24) as a central element of the disease rather suggests an increased susceptibility towards hazardous stimuli of the skin in patients with vitiligo. However, this concept did not clarify what activates the immune system in the first place, and why melanocytes are the main target.

Yet another question is whether the observed alterations could be the consequence of a more generalized impairment. On the basis of our results, we speculate that the membrane plays a central role. The lipid composition and the insufficient saturation of fatty acids compromise the membrane tridimensional structure, giving rise to altered intramembrane protein assembly on the plasmatic as well as on the mitochondrial membrane (25). Consequently, receptor activity and activity are affected (26–28). Lipid remodelling is an important mechanism for targeting proteins within cells and for controlling their activity. The fluidity of the membrane is due to the cholesterol content and the pattern of fatty acids of the phospholipids. Cholesterol ensures the correct structure and function of the glycolipid- and cholesterol-enriched *lipid rafts*, where the receptors, and the associated protein kinases, can move and cluster. The