

example, antigens released from pigment cells disrupted by non-immune mechanisms could initiate immune reactivities that target melanocytes which then exacerbate the disorder. Indeed, interestingly, melanocyte-specific cytotoxic T lymphocytes and autoantibodies have been identified in patients with melanoma where vitiligo has occurred during immunotherapy with specific pigment cell antigens (15,16). Of course, melanocytes could also come under attack from immune responses if they expose antigens that are similar to either an infectious agent or to other cells that are themselves the primary target of the autoreactive T cells or autoantibodies.

Alongside the autoimmune hypothesis, the theory that metabolic deregulation can lead to the production of toxic metabolites that damage melanocytes resulting in vitiligo is well documented and supported by much evidence. The biochemical hypothesis argues that the destruction of melanocytes is due to the accumulation of toxic metabolites from melanogenesis, the break down of free-radical defense and an excess of hydrogen peroxide (17–20). Notably, new *in vitro* studies have provided a link and a temporal sequence connecting cellular oxidative stress and the immune response in vitiligo: stressed melanocytes were found to mediate dendritic cell activation with the consequent dendritic cell effector functions playing a role in the destruction of pigment cells. This work suggests that intrinsic damage to pigment cells could be the initiating event in the development of vitiligo followed by a secondary immune response which exacerbates the destruction of melanocytes and progresses the disease (21) and as such supports the proposed convergence theory (1).

Undoubtedly, the genetic influence on the development of vitiligo is strong and evidence for the contribution of biochemical defects and autoimmunity on the development

of the disease is substantial. Overall, further research regarding the aetiology of vitiligo is required to determine the exact contribution that different factors make to initiation of the disease. This should lead to an improvement in therapeutic modalities and will also aid in setting the criteria for clinical classification of the disease as different pathogenic mechanisms could account for the various clinical types of vitiligo (2).

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Commentary 2

Vitiligo is an acquired depigmentation disorder affecting 0.5–2% of the world population. Multiple theories of pathogenesis exist, and some support exists for an autoimmune aetiology (1). This hypothesis proposes that an immune system disorder results in the destruction of melanocytes. It is first supported by the frequent observation that several autoimmune disorders (thyroid diseases, Addison's disease, etc.) are associated with vitiligo. A significant association of vitiligo was demonstrated with thyroid dysfunction and/or thyroid antibodies in particular (1).

Concerning humoral immunity, antibodies to surface and cytoplasmic antigens of melanocytes have been found in patients with vitiligo, mainly belonging to the IgG class. The autoantigens most frequently identified are antigens related to HLA class I molecules, tyrosinase, tyrosinase-related protein (TRP)-1 and TRP-2 (the last three are

melanocyte-specific antigens) (2). However, the pathogenic role of antimelanocyte antibodies remains unclear. The serum levels of antibodies to melanocyte antigens seem to correlate with activity and extent of the disease and with the presence of other immune disorders, and to decrease in patients with vitiligo responding to therapy (2).

A very recent and large epidemiological study supports the involvement of both genetic and non-genetic factors in the pathogenesis of the disease (3). A positive family history for vitiligo is reported. Actually, family clustering of cases is not uncommon, as about 20% of patients have at least one affected first-degree relative, with a non-Mendelian pattern suggestive of multifactorial, polygenic inheritance (4); segregation analyses suggest the involvement of multiple interacting genes in different populations (5). Several genes and chromosomal regions have been implicated

in susceptibility to vitiligo, but none has been confirmed so far (5). In addition, several HLA abnormalities have been associated with vitiligo, including association with Dr4, B13, BW35 and A30 (6).

Recently, one of the major hypotheses in the pathogenesis of vitiligo is the oxidative stress hypothesis. It has been shown *in vivo* and *in vitro* that patients with vitiligo accumulate high levels (mM) of hydrogen peroxide (H_2O_2) in their epidermis (7). In the past, several sources for this unusual epidermal H_2O_2 production/accumulation have been documented. Moreover, it is also well established that millimolar levels of H_2O_2 lead to the inactivation of catalase and in this context it is noteworthy that low epidermal as well as systemic catalase levels have indeed been described in this patient group (7–14). Table 1 summarizes the sources of H_2O_2 documented to date in vitiligo (15).

The clinical hallmark of vitiligo is the loss of the inherited skin colour in association with a characteristic fluorescence under Wood's light (351 nm) examination (8). This fluorescence has been attributed to the accumulation of 6- and 7-biopterin as well as to the formation of pterin-6-carboxylic acid via H_2O_2 oxidation (16). Moreover, it was shown that the epidermal *de novo* synthesis/recycling/regulation of the essential cofactor (6R)-L-erythro-5,6,7,8-tetrahydrobiopterin (6BH₄) is perturbed in vitiligo (8,13). Here, it is noteworthy that 6BH₄ is the immediate electron donor for the hydroxylation of the aromatic amino acids L-phenylalanine, L-tyrosine and L-tryptophan (17). Both epidermal melanocytes and keratinocytes hold the full capacity for autocrine 6BH₄ *de novo* synthesis/recycling and regulation (18,19).

Figure 1 presents a simplified scheme for the *de novo* synthesis/recycling/regulation of this cofactor with its

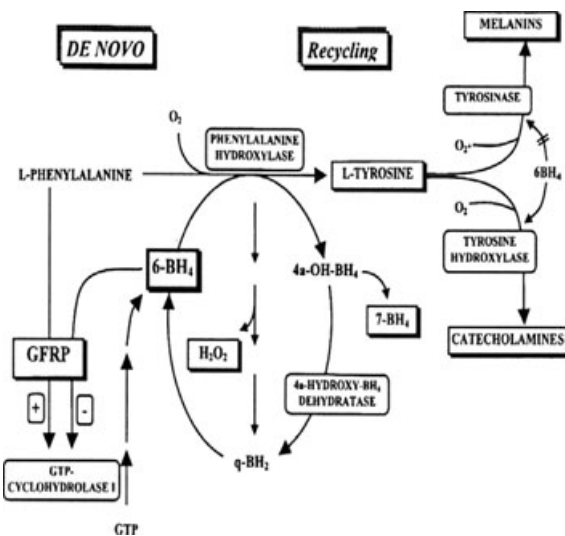


Figure 1. *De novo* synthesis/recycling/regulation of 6BH₄ in the production of L-tyrosine as a substrate for melanogenesis and catecholamine synthesis.

impaired metabolic steps in vitiligo. Defective calcium homeostasis is also observed in patients with vitiligo, that in turn affects the redox status of 6-biopterin/tetrahydrobiopterin equilibrium, monoamine oxidase activity among others (20,21). Moreover, it was demonstrated that removal/reduction of epidermal H_2O_2 in patients with acute vitiligo by a pseudocatalase PC-KUS caused restoration and upregulation of the above enzyme protein expression and activities in association with restoration of the original skin colour (9).

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Table 1. Confirmed sources for epidermal/systemic H_2O_2 generation/accumulation in vitiligo

Source (reference)	H_2O_2 generation/accumulation	Change
Monoamine oxidase A (10)	E	↑
NADPH oxidase (12)	E	↑
Photo-oxidation of pterins (16)	E	↑
Nitric oxide synthases (22)	E	↑
Short circuit in the 6BH ₄ recycling (8)	E	↑
Catalase (7)	B, E	↓
Glutathione peroxidase/glutathione (23)	B	↓
Tyrosinase-related protein 1 (24)	E	↓

↑, increased; ↓, decreased; E, in epidermis; B, in blood.

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Commentary 3

Mannose-binding lectin (MBL) is one of the infamous foot soldiers that defend the body against micro-organisms in the never-ending ‘trench warfare’ fought within the body’s perimeter. It fights on four different fronts: (1) complement activation; (2) opsonophagocytosis; (3) modulation of inflammatory response; and (4) the promotion of apoptosis (1–3). Poorly equipped or insufficient numbers of soldiers are not capable of operating effectively and allow attacking micro-organisms to infiltrate the body which trigger a series of events resulting in the destruction of the body (autoimmune diseases) as is stated by Werth *et al.* Cleaning up the battlefield (clearance of the apoptotic cells) after the war between the micro-organisms and the immune-competent cells is one of the soldiers’ main functions (4). Inefficient clearance leads to a harmful autoimmune response (5,6).

The MBL infantry plays a role in controlling the body’s overreactions represented by proinflammatory cytokines and the clearance of immune response remnants, such as immune complexes and adhesion molecules on inflammatory cells, which often lead to casualties on both sides (7,8).

There have been a number of reports showing associations between MBL deficiency and autoimmune disorders such as systemic lupus erythematosus (SLE), dermatomyositis and cutaneous lupus erythematosus (4,9–11).

In the process of vitiligo, there may be a specific antigenic stimulus which initiates the defensive actions of immune-competent cells in the skin. During these actions, a number of immune-competent skin cells go into apoptosis resulting in alterations of membrane carbohydrates. MBL has the ability to bind to these altered carbohydrates and to facilitate the clearance of apoptotic cells (4,6). Inadequate clearance of the apoptotic cells may cause continuous stimulation of the immune system and antibody production resulting in vitiliginous changes in the skin cells (Fig. 1).

It has also been shown that there is insufficient calcium (Ca) uptake in melanocytes and keratinocytes from vitiliginous skin (12,13).

The infantry’s (MBL) command and supply chain rely heavily on Ca uptake. We hypothesize that a weak supply and command chain (Ca) and insufficient infantry (MBL) may contribute to undesirable consequences of the war (vitiligo). Therefore, we consider that sufficient numbers of infantry (normal MBL alleles/levels) provide a strong level of protection on the war against vitiligo.

A reduced infantry may also result in a predisposition to attack from specific viral agents such as cytomegalovirus, Epstein–Barr virus, hepatitis E, and of course, the AIDS virus on weakened war fronts (14,15). For example, Grimes *et al.* identified cytomegalovirus DNA in skin biopsy specimens of patients with vitiligo (16).

All of these notions support the idea that MBL deficiency may play an important role in susceptibility to vitiligo and replacement of deficient MBL may be helpful in combating vitiligo lesions and/or prevent its progression.

Recently, recombinant human MBL (rMBL) has been produced (17) and a phase I study has been designed to evaluate the safety, tolerability, pharmacogenetic and immunogenetic profile of rMBL (18,19). In one of these, this phase I study no adverse effects of intravenous rMBL were observed in healthy but MBL deficient males (18). Protective role of mannose-binding lectin in a murine model of invasive pulmonary aspergillosis was also shown by Kaur *et al.* (20).

Regarding the protective roles of normal MBL levels in patients with vitiligo, we propose that patients be treated with a topical application of a gel or pomade (name of it can be ‘vitiliMBL’) containing rMBL which is already available (17). We also consider the addition of Ca to this pomade or/and an increased Ca intake to be even more effective in the treatment of vitiligo.

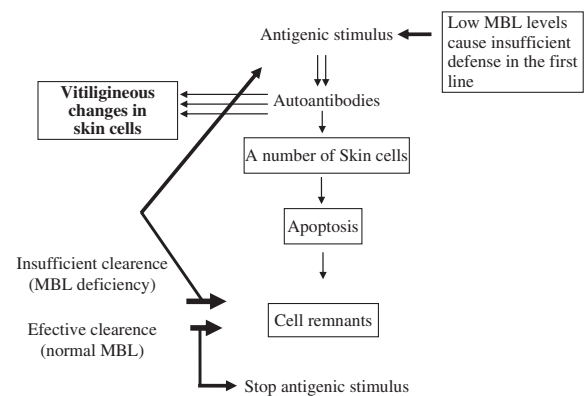


Figure 1. In the aetiopathogenesis of vitiligo, low serum MBL levels may be associated with the impaired defense in the first step of immune response and insufficient clearance of apoptotic cells resulting in autoimmune reactions in the skin cells.