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Surgical Management of Vitiligo
Dedicated to my parents, Dr. Ram Pratap Gupta and Indra Gupta, who nurtured my interest in Medicine, to my wife Dr. Meenu who was with me all along the way, and to my yet unborn children (twins) through whom I see my vision for the future.

Somesh Gupta

To the patients and to my most highly respected friends, colleagues, and mentors Dr. Aaron B. Lerner (Yale University) and the late Dr. Lennart Juhlin (Uppsala University) for always supporting my thoughts and development and for an absolutely reliable friendship.

Mats J. Olsson

Dedicated to vitiligo patients

Amrinder J. Kanwar
Surgical Management of Vitiligo

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Foreword

To raise new questions, new possibilities, to regard old problems from a new angle, requires creative imagination and marks real advance in science.

Albert Einstein

Many researchers throughout the world have dedicated their time and lives in search of the etiology of vitiligo, an important skin pathology that although physically asymptomatic in most patients, provokes profound changes in psychological behavior and social interaction with other individuals in daily life. And most important, in spite of multiple known factors involved in its pathogenesis that have been published in many journals devoted to different disciplines – from the laboratory to the ambulatory patient – finding the cause of this condition has been elusive.

To our satisfaction, remarkable progress has been made in the past two decades. When medical therapy is no longer useful for treating depigmented lesions, melanocyte transplantation, performed judiciously, provides acceptable repigmentation for an important proportion of appropriately selected patients presenting with stable disease.

We are fortunate that Professors Somesh Gupta, Mats J. Olsson, Amrinder J. Kanwar and Jean-Paul Ortonne have provided the readers with a superb book consisting of 38 chapters written by 48 international authorities from 13 countries, in their fields of expertise. This magnificent book is the most comprehensive scientific work written to date, in which all topics of surgical management of vitiligo are covered by a panel of well known authors. Not only are all pertinent surgical topics dealt with in fine detail, but also the current understanding of pathogenic mechanisms and clinical aspects of the disease are described, providing the foundations for determining which patients may be candidates for surgical repigmentation therapy.

Finally, after reading the contents and concepts of Surgical Management of Vitiligo, I was inspired to express the following words: “Innovation increases human knowledge though sometimes originates controversy, which in turn stimulates improvement.”

Rafael Falabella, MD
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The concept of surgically treating vitiligo was first proposed by a few pioneers in the 1960s. Over the years, this technique has developed considerably and is becoming progressively more sophisticated.

Vitiligo is characterised by a lack of melanocytes, which are destroyed by various unidentified mechanisms. Initial attempts to repigment vitiligo skin were aimed at mobilising melanocytes from unaffected skin and/or hair follicles to depigmented skin lesions. This strategy, which is often tedious, does not consistently provide the desired results, even though a little more is now known about the mechanisms that control the migration and differentiation of melanocyte precursors found in hair follicles.

Several autologous pigmented skin graft techniques for vitiligo patients have been developed in order to facilitate the proliferation and migration of melanocytes in grafts so that they colonise the vitiligous skin in which they were implanted. These techniques were micronised in order to reduce and avoid any skin abnormalities that could result from the healing of these grafts. They can be used for mucous membranes and on sensitive areas such as the eyelids, and areas that are resistant to repigmentation, such as the fingers. The transplantation of pigmented hair follicles to vitiligous skin has also been successfully explored.

Cellular grafts of epidermal cell suspensions (keratinocytes and melanocytes) or autologous melanocytes cultivated prior to grafting have also been used successfully. Several groups even suggest grafting epidermis that is reconstructed in vitro, consisting of both melanocytes and epidermal keratinocytes.

What is the future for the surgical treatment of vitiligo? It is likely that these “biotherapies” will become part of future vitiligo treatments, either by using cytokines, or growth factors that facilitate the differentiation, proliferation and migration of melanocytes. Melanocyte progenitors and/or human embryonic stem cells will most likely have a role in future vitiligo biotherapies, to the extent that the tolerance and efficacy of factors that modulate pigment cells will have been demonstrated. Also, a U.S. team has recently reported that it is possible to grow functional melanocytes from human embryonic stem cells in vitro. One can therefore hope that the production of melanocytes for the repigmentation of vitiligous skin will become easier and more productive in the future.

Cellular graft techniques also need to evolve. It is currently possible to prepare the ‘bed’ for melanocyte grafts by destroying the epidermis using lasers, while avoiding damage to the basal membrane at the epidermal-dermal junction. It is hoped that future techniques will improve this step further.

I would like to thank Somesh Gupta and Amrinder J. Kanwar for inviting me to be involved in the publication of this book with Mats J. Olsson, one of the pioneers in melanocyte grafting for the treatment of vitiligo. The resulting monograph, the first publication that is dedicated to the surgical treatment of vitiligo, is a true encyclopedia containing contributions from most of the global teams.
that surgically treat vitiligo. It will not be the last and should be regularly updated as progress is made in this area.

I hope you enjoy reading it and I hope it meets your expectations. Perhaps in the future, effective therapies that block the processes that cause skin depigmentation will become available. In the meantime, we need to continue the progress being made on repigmenting techniques. For several skin sites, surgical and biotherapeutic approaches will be the optimal choice.

Jean-Paul Ortonne, MD
SECTION 1

Pathogenesis and medical treatment
CHAPTER 1
Pathogenesis of vitiligo

Jean-Paul Ortonne

Introduction
Vitiligo is an acquired cutaneous hypomelanosis with a 0.5–2% incidence worldwide, without predilection for sex or ethnicity. The clinical presentation is characterized by well-circumscribed white macules. Several clinical phenotypes have been identified. Generalized vitiligo is characterized by acquired depigmentation due to melanocyte loss, in a pattern that is non-focal and generally bilateral across the midline, though not necessarily symmetric [1]. This definition differentiates generalized vitiligo from segmental vitiligo and other localized forms of vitiligo whose true pathogenic relationship to generalized vitiligo is as-yet unknown.

This chapter is a review of the recent developments of vitiligo pathogenesis. There are three major hypotheses for the pathogenesis of vitiligo that are not exclusive of each other – the autoimmune hypothesis, the neuronal dysfunction hypothesis, and the melanocyte self-destruction hypothesis. Several other hypotheses have been recently proposed.

Vitiligo: a melanocyte disorder or more?
Melanocytes present or absent in vitiligo macules?
Vitiligo is characterized by a disappearance of epidermal and/or follicular melanocytes. It is likely that melanocytes are destroyed by an as-yet unknown process. Indeed, melanocyte destruction has never been clearly demonstrated [2]. One recent study reports that melanocytes are never completely absent in the skin [3]. Melanocyte cultures were successfully established from depigmented epidermal suction blister roof of 12 randomly selected vitiligo patients. These “vitiligo” melanocytes produced melanin in vitro. Although interesting, these observations in a small group of patients cannot be generalized. The persistence of melanocytes within vitiligo macules was already reported in 1956 [4]. Besides the so-called “absolute” type of vitiligo in which there are no dopa-positive melanocytes in the vitiliginous epidermis, there are “relative” types of vitiligo in which melanocytes remain in the white macules with a decreased dopa-positivity. It is likely that the disappearance of epidermal melanocytes in vitiligo macules is not an immediate process, but is a progressive one. Indeed, it may be suggested that the “relative” types of vitiligo are considered possible forerunners of the “absolute” types. Thus, it would not be surprising to find melanocytes in the epidermis of the white macule of “relative” vitiligo. An immunohistological study of vitiliginous skin using a panel of melanocyte markers, related and unrelated to the melanogenic pathway, could not detect identifiable epidermal melanocytes [5]. From the presently available data, it is generally agreed that there are no longer functional melanocytes in vitiligo skin and that this loss of histochemically recognizable melanocytes is the result of their destruction.

The impact of vitiligo on melanocyte stem cells (MSC) is not known. MSC are present in the bulge region of hair follicles in the adult skin in mice. Undifferentiated MSCs in the bulge have been shown to express the three transcription factors, PAX 3, SOX 10, and MITF. These factors play a key role in controlling the balance between MSC maintenance and differentiation. Studies to evaluate MSCs in vitiligo-pigmented and depigmented hair follicles are strongly required to better understand the mechanism of poliosis in vitiligo patients and

3
the cellular event underlying perifollicular repigmentation of vitiligo [6].

**Keratinocytes**

Several observations suggest that epidermal cells other than melanocytes are also altered in vitiligo involved and uninvolved skin. Epidermal keratinocytes produce several factors that support the growth and differentiation of neighboring melanocytes, such as basic fibroblast growth factor (b-FGF) and stem cell factors (SCF). A recent study demonstrated that the expression of SCF \((P < 0.001)\) and b-FGF was usually reduced in the depigmented compared with the normally pigmented vitiligo epidermis [7]. SCF has been demonstrated to prevent TRAIL-induced melanocyte apoptosis in vitro. These results suggest that melanocyte cell death in vitiligo can result from deprivation of keratinocyte-derived SCF.

Cytoplasmic vacuolization and/or the presence of an extracellular granular material that may be derived from the cytoplasm of altered keratinocytes have been reported mainly in the adjacent normal-appearing vitiligo skin, but also in the perilesional skin and rarely in the lesional skin [8]. Focal areas of vacuolar degeneration in the lowest layers of the epidermis, especially in the basal layer in association with mild mononuclear cell infiltrate, have also been observed [9].

The significance of these morphological observations is not known, but several hypotheses can be proposed. They may be related to architectural disturbances induced by a local immunological reaction. They may be due to toxic intermediate metabolites of melanogenesis, which destroy not only the pigment cell from which they originate [8], but also the adjoining keratinocytes. The recently proposed theory of a breakdown in the detoxification mechanisms in vitiligo skin fits very well with these observations.

**Langerhans cells**

The role of Langerhans cells in vitiligo has been open to controversy. The Langerhans cell density evaluated either by histochemical techniques (ATPase) or with the monoclonal antibodies OKT6 and anti-HLADR has been variably reported as decreased, normal, or increased [10]. In addition to these quantitative changes of Langerhans cells, a functional impairment of these cells has also been documented in vitiligo skin. How this functional impairment of Langerhans cells is related to the pathogenesis of vitiligo remains to be established.

All these observations suggest that vitiligo affects the entire keratinocyte–Langerhans cell-melanocyte unit (KLM) [11]. In the epidermis there is a complex exchange of messages between these three cell types that is just beginning to be understood. No doubt a better clarification of these epidermal cell interactions will help in understanding the basic mechanisms involved in vitiligo.

**Genetics of vitiligo**

**Epidemiological data**

Familial studies have shown the increased prevalence of vitiligo in close relatives of affected individuals. In a large series performed in India, this increase was about 4.5-fold in close biological relatives [12]. Another study performed on 160 white kindred living in US shows a relative risk (RR) for vitiligo of about 7 for parents, about 12 for siblings, and about 36 for children [13]. The pattern of relationship between RR and degree of kinship indicates involvement of genetic factors, although it is not consistent with single-locus Mendelian transmission. The major genetic component in vitiligo pathogenesis and also the role of environmental factors were recently emphasized [14]. In this epidemiological study the frequency of vitiligo in probands’ siblings was 6.1%, about 18 times that of the population frequency. Nevertheless, the concordance of vitiligo in monozygotic twins was only 23%, indicating that a non-genetic component also plays an important role. Moreover, probands with earlier disease onset tended to have more relatives affected with vitiligo, suggesting a greater genetic component in early onset families.

**One vitiligo or several vitiligos?**

For most authors, vitiligo is a unique disorder with several clinical presentations but one physiopathology. Indeed, almost all the recent genetic studies have ignored the clinical presentation of patients. However, recent data strongly suggest that there is not one
vitiligo but several vitiligos. A complex segregation analysis was performed on 2247 Chinese patients and their families. For the first time the results were analyzed according to the clinical manifestations [15]. The results showed a different age of disease onset depending on the subtypes of vitiligo. More interestingly, a polygenetic additive model was found to be the best model for segmental, localized, acrofacial, and generalized vitiligo whereas the best model for universal vitiligo was an environmental model. All of these data suggest that heterogeneous pathogeneses underlie different phenotypes of vitiligo.

**Genetic aspects of vitiligo**

The earliest genetic studies of vitiligo were case-control association studies of the major histocompatibility complex (MHC). They were carried out by testing various different vitiligo phenotypes versus controls in many different populations. Genetic association of vitiligo with alleles of MHC loci appeared to be strongest in patients and families with various vitiligo-associated autoimmune/autoinflammatory disorders versus patients and families with only generalized vitiligo. Thus, it is not clear whether the MHC association is with vitiligo, vitiligo-associated autoimmune/autoinflammatory disorders, or both.

Allelic association between vitiligo and a number of other candidate genes has also been described (Table 1.1).

**Table 1.1 Susceptibility loci for vitiligo.**

<table>
<thead>
<tr>
<th>References</th>
<th>Susceptibility locus</th>
<th>Mapping</th>
</tr>
</thead>
<tbody>
<tr>
<td>[73]</td>
<td>SLEV1</td>
<td>17p13</td>
</tr>
<tr>
<td>[18]</td>
<td>AIS1</td>
<td>1p31.3–p32.2</td>
</tr>
<tr>
<td>[18]</td>
<td>AIS2</td>
<td>7p</td>
</tr>
<tr>
<td>[18]</td>
<td>AIS3</td>
<td>8q</td>
</tr>
<tr>
<td>[74]</td>
<td></td>
<td>6p21.3–21.4</td>
</tr>
<tr>
<td>[75]</td>
<td></td>
<td>4q13–q21</td>
</tr>
</tbody>
</table>


**Which gene(s) for vitiligo?**

Two large genome-wide screens for generalized vitiligo showed significant linkage of an oligogenic autoimmune susceptibility locus, termed AIS1 (1p31.3–p32.2) [16,17]. An additional seven signals on chromosome 1,7,8,11,19, and 22 met genome-wide criteria for “suggestive linkage.” In an extended study with a cohort of 102 multiplex families the localization of AIS1 was confirmed and two new susceptibility loci have been found. AIS2 is located on chromosome 7 and AIS3 on chromosome 8. Additionally, the locus SLEV1 on chromosome 17 was confirmed and two new potential linkages on chromosome 9q and on 13q are also reported (Table 1.1) [18]. Interestingly, all loci except AIS3 derive principally from the autoimmunity-associated family subgroup. These loci may predispose to a vitiligo-associated autoimmunity diathesis. On the other hand, analyses suggest a linkage to SLEV1 in the autoimmune families and non-linkage in the non-autoimmune families. Thus, linkage to SLEV1 in these families indicates that SLEV1 confers susceptibility to a broader range of autoimmune diseases than just lupus and vitiligo. A genome-wide linkage analysis in Chinese families identified interesting linkage evidence in 1p36, 4q13–21, 6p21–p22, 6q24–q25, 14q12–q13, and 22q12. These findings in the Chinese population shared a minimal overlap with the linkage findings in the Caucasian population. Such little overlap between the linkage findings of these two populations may suggest that vitiligo is associated with a strong genetic heterogeneity [19].

Many candidate genes for vitiligo have been proposed so far (Table 1.2). However, most of the loci described do not correspond to positions of these proposed biological candidate genes.

Finally, one of the best candidate genes could be FOXD3 (“Forkhead box” D3). FOXD3 is located on chromosome 1 (1p32–p31) and is a transcription factor that suppresses melanoblast development from the neural crest [20]. Therefore, dysregulated (over-)expression might harm melanocytes. Moreover FOXD3 also regulates endodermal differentiation including thyroid, pancreas, adrenal, and gut [21] and other FOX factors are involved in autoimmune syndromes [22]. Mutations in FOXD3
leading to elevated FOXD3 transcription have been recently reported in one AIS1-linked family [23]. Thus, FOXD3 is worth further investigation and represents a serious candidate gene in AIS1-linked autoimmune disease.

Pathogenesis

The classic hypotheses

Vitiligo is an autoimmune disease

This theory is the most long-standing and popular hypothesis for the pathogenesis of vitiligo. It proposes that melanocytes are killed by autoimmune effector mechanisms.

Association with autoimmune disease

Sporadic generalized vitiligo is associated with autoimmune thyroid disease, pernicious anemia, Addison’s disease, systemic lupus erythematosus [14]. Familial generalized vitiligo is also characterized by a broad repertoire of associated autoimmune diseases, such as thyroiditis, rheumatoid arthritis, psoriasis, adult-onset-dependent diabetes mellitus, pernicious anemia, and Addison’s disease [24]. Furthermore generalized vitiligo is a component of the APECED (APS1) and Schmidt (APS2) multiple autoimmune disease syndromes. These same vitiligo-associated autoimmune/autoinflammatory disorders also occur, at increased frequencies, in patients’ first-degree relatives, regardless of whether or not those relatives have vitiligo themselves. These observations suggest that specific genes predispose to a specific group of autoimmune diseases that includes generalized vitiligo, autoimmune thyroid disease, rheumatoid arthritis, psoriasis, adult-onset insulin-dependent diabetes mellitus, and pernicious anemia.

Cellular immunity

Recent evidence has emerged for a role for cell-mediated immunity in vitiligo pathogenesis. The
discovery of a T-cell infiltrate in the margin of inflammatory vitiligo was the first clue for participation of cellular immunity in vitiligo pathogenesis. Infiltrating activated CD4 and CD8 T-cells, but not the B-cells, have been observed at the periphery of vitiligo lesions [25]. A more recent study of vitiligo lesional skin noted a high frequency of cutaneous lymphocyte antigen-positive-activated cytotoxic T-cells clustered in perilesional skin in the vicinity of disappearing melanocytes [26]. Furthermore, melanocytes in close proximity to activated lymphocytes locally expressed HLA-DR and intercellular adhesion molecule-1, suggesting a major role for skin-homing T-cells in melanocyte death [26]. The reports of an increase of CD45RO memory T-cells, increased levels of soluble interleukin-2 receptors and expression of the cutaneous lymphocyte antigen in number infiltrating T-cells, all suggest an activation of circulating T-cells and their recruitment to the vitiligo skin [27–29]. In vitiligo skin the CD4/CD8 ratio is reversed with a predominant presence of CD8 T-cells.

Further evidence for a role played by cytotoxic T-cells in vitiligo stems from studies of melanoma patients. Vitiligo-like depigmentation has been observed following successful immunotherapy of melanoma, including high-dose IL-2 therapy, infusion of peptide pulsed dendritic cells, and Melan A/MART-1 specific CTL clones [30]. A specific cellular immune response predominantly directed against the melanosomal protein Melan-A/MART-1 was observed in HLA-A2-positive vitiligo patients where CD8+ T-cells displaying Melan-A/Mart-1-specific reactivity ex vivo were demonstrated in the peripheral blood of these patients. Another study reported evidence of an association between CD8 + T-lymphocyte reactivity to the melanocyte antigen gp100 and to a lesser extent Melan-A/MART-1 and vitiligo [30]. These findings support the concept of an immunopathological mechanism in vitiligo in which cell-mediated play a crucial part. Recently, new understanding for the requirements for CD8+ T-cell mediated destruction of melanocytes was brought [31]. CD4+ T-cell help induced by systemic immunization and a local inflammation are both required to break MHC class-I-restricted T-cell tolerance.

### Table 1.3 Identified target autoantibodies for vitiligo antibodies.

<table>
<thead>
<tr>
<th>Autoantigen</th>
<th>Function</th>
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<tbody>
<tr>
<td>Tyrosinase</td>
<td>Melanogenic enzyme</td>
</tr>
<tr>
<td>TRP-2</td>
<td>Melanogenic enzyme</td>
</tr>
<tr>
<td>TRP-1</td>
<td>Melanogenic enzyme</td>
</tr>
<tr>
<td>Pmel-17</td>
<td>Melanocyte-specific protein</td>
</tr>
<tr>
<td>MCHR1</td>
<td>Melanin concentrating hormone receptor 1</td>
</tr>
<tr>
<td>SOX 9</td>
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### Humoral immunity

Several circulating autoantibodies (Table 1.3) have been found in sera of vitiligo patients. These include antibodies to non-pigment cell antigens (common tissue antigens), cytoplasmic pigment cell antigens, and pigment cell surface antigens [32]. The heterogeneity of this antibody response is surprising and does not fit with a selective destruction of melanocytes. One reasonable explanation is that this humoral response could be secondary to a primary melanocyte destruction mediated by other mechanisms.

The incidence and serum level of antibodies was found to correlate with the disease activity and the extent of the cutaneous depigmentation. Functional in vitro assays have shown the ability of antibodies to damage melanocytes, both by complement activation and by ADCC [33]. Furthermore injections of IgG fractions of serum from patients with vitiligo have a destructive effect on melanocytes of the human skin grafted onto nude mice [34].

Antibody-dependent immunity against the melanosomal membrane protein-1 (TYRP-1) of melanocytes leads to autoimmune hypopigmentation. Hypopigmentation occurred in mice deficient in activating FcR containing the common γ subunit and in mice deficient in the C3 complement but not in mice doubly deficient in both FcγRγ and C3 [35].

### The neural hypothesis

There are several observations, clinical findings, and laboratory evidence that suggest the involvement of the nervous system in the pathogenesis of

Pathogenesis of vitiligo
vitiligo. Embryologically, melanocytes are derived from the neural crest. There are isolated reports of vitiligo associated with viral encephalitis and transverse myelitis. Communication between the nervous system and epidermal melanocytes has been proved [36]. Ultrastructural studies demonstrate frequent direct contacts between dermal nerve endings and melanocytes in vitiligo skin [37] or structural alterations (swelling of axons, duplication of the basement membrane, etc.) [38,39], but the significance of these morphological findings is unknown. The neural hypothesis is based in the first place on the presence of segmental vitiligo. The distribution of segmental vitiligo is often said to be dermatomal, suggesting the role of regional nerves in this condition. In actuality it is unilateral, but not dermatomal (i.e. it does not follow a specific pattern of cutaneous sensory nerves) [40].

Thus, the role of the nervous system in the pathogenesis of vitiligo is still undefined.

A few physiological studies have demonstrated altered bleeding times, epinephrine vasoconstrictor effect, and abnormal sympathetic skin responses in lesions of vitiligo [41,42]. Other studies have shown altered neuropeptides in vitiligo. Aberrations in β-endorphin and met-enkephalin secretion have been reported [43]. The plasma met-enkephalin levels were generally higher in vitiligo patients, especially in those with active vitiligo, than in controls. Immunohistological observations suggest that the immunoreactivity to neuropeptide Y and vasoactive intestinal polypeptide is increased at the marginal areas or within vitiligo macules. These observations support the hypothesis of neural involvement and neuro-immunomodulation in vitiligo [39]. Still other studies have demonstrated a reduction in the immunoreactive nerve growth factors (NGFr-IR) [44] and an absence of Merkel cells [45].

Several studies demonstrating abnormalities of acetylcholine, catecholamines, or related enzymes (catechol-o-methyltransferase (COMT) and monoamino oxidase [46,47]) have also been reported with conflicting results [48–50]. A reduced acetylcholinesterase activity in vitiliginous skin as compared to adjacent normal skin has been reported [51]. Increased urinary levels of catecholamines have been found during the active phase of vitiligo [52,53].

The autocyctotoxic theory

In 1971, Lerner [54] postulated that melanocytes have a genetically based protective mechanism that eliminates toxic products like DOPA, DOPAchrome, and 5,6-dihydroxyindole, manufactured during melanogenesis. Individuals who are deficient in this mechanism have accumulation of these melanotoxic products, which results in depigmentation. Another possible mechanism could be damage by genetic mechanisms or by perioxidation [55] to the membranes of melanosomes, which prevent leakage of these compounds into the cellular milieu [56,57].

The oxidative stress theory proposes that melanocyte death results from an intrinsic increased sensitivity to oxidative stress either from toxic intermediates of melanin precursors or from other sources. In vivo and in vitro evidence for hydrogen peroxide ($\text{H}_2\text{O}_2$) accumulation in the epidermis of vitiligo patients has been reported, resulting from low epidermal catalase levels [58]. Several studies suggest that (a) cultured vitiligo melanocytes exhibit increased sensitivity to oxidative stress and (b) catalase helps to establish vitiligo melanocyte cultures and to restore melanocyte functions after exposure to $\text{H}_2\text{O}_2$ [2]. According to Schallreuter and her group, the origin of the epidermal $\text{H}_2\text{O}_2$ accumulation and low epidermal catalase levels within the entire skin of vitiligo patients may arise from several potential sources: (1) perturbed (6$\text{R}$)-l-erythro-5,6,7,8-tetrahydrobiopterin (6BH4) de novo synthesis/recycling/regulation; the absence of catalase leads to accumulation of toxic superoxide radicals. One such mechanism involves a group of compounds called pteridines. L-tyrosine is the central substrate for catechol synthesis in keratinocytes and melanin synthesis in melanocytes L-tyrosine itself is produced from L-phenylalanine and the reaction is regulated by the enzyme phenylalanine hydroxylase. This enzyme is under the control of pteridines including 5,6,7,8-tetrahydrobiopterin (6BH4). Defects in the production of pteridines lead to the accumulation of epidermal phenylalanine and shortage of L-tyrosine. A clinical study using a loading oral dose of L-phenylalanine showed slower turnover of L-phenylalanine to L-tyrosine in patients compared with controls [59]. The defective deranged synthesis of pteridines leads to the concomitant accumulation of $\text{H}_2\text{O}_2$;
(2) impaired catecholamines synthesis with increased mono-oxygenase A activities; (3) low glutathione per-oxidase activities; (4) "oxygen burst" via NADPH oxidase from a cellular infiltrate [58]. Under in vitro conditions, vacuole of vitiligo melanocytes has been demonstrated which was reversible upon exogenous addition of bovine catalase to the culture medium [3]. Until now, this interesting concept has not yet been validated in vivo in patients with vitiligo. Whether H$_2$O$_2$ is the cause or the consequence of vitiligo remains to be identified. Another mechanism involves the ion calcium. In the epidermis there is an efficient antioxidant mechanism of thioredoxin/thioredoxin reductase (T/TR) which reduces H$_2$O$_2$ to water. The activity of this system is allosterically regulated by ionic calcium and defective uptake of calcium could result in altered redox status and accumulation of H$_2$O$_2$ [60]. Estrogens can also contribute to hydrogen peroxide [61]. Successful removal by a UVB-activated pseudocatalase has also been reported. However, this conclusion arises from an open trial including only 33 patients and the results have not been confirmed by further studies. The efficacy of pseudocatalase to promote vitiligo repigmentation is still a matter of debate. However, there is no abnormality in blood antioxidant status in patients with vitiligo. Blood levels of superoxide dismutase, glutathione peroxidase, glutathione reductase, non-enzymatic oxidants such as α-tocopheryl (Vit E), retinol (Vit A), ascorbic acid (Vit C) have been found to be normal.

In the context of the oxidative stress theory, selenium is widely prescribed to stabilize and to repigment vitiligo. However, two distinct studies demonstrated that there is an increase in total blood antioxidant status (high serum selenium levels) in vitiligo patients [62,63]. As a consequence, oral supplementation should not be practiced in patients who exhibit a spontaneous increase in selenium levels, as it could be potentially harmful (selenium toxicity).

The new hypotheses

A disorder of melanocyte survival

The active mechanism by which melanocytes are destroyed in vitiligo skin has not yet been determined. Several morphological observations suggest the involvement of melanocyte apoptosis and of the SCF/c-kit/MITF/Bcl-2 pathway in the pathogenesis of vitiligo. This pathway plays a key role in the maintenance of melanocyte survival. SCF of keratinocyte origin strongly protects melanocytes from TNF-related apoptosis inducing ligand (TRAIL) [64]. Bcl-2, a MITF-dependent kit transcriptional target in melanocytes, is essential for the maintenance of an appropriate lifetime for melanocytes. The decrease of Bcl-2 expression of melanocytes increases their susceptibility to apoptosis. Bcl-2$^{-/-}$ mice develop graying and whitening of hair early in life during the second hair cycle, due to disappearance of follicular melanocytes. Levels of SCF expression ($P < 0.001$) are reduced in the depigmented epidermis of vitiligo patients compared to normally pigmented paired epidermis [7].

A reduction in the number of kit-positive melanocytes in the perilesional skin of vitiligo patients has also been reported. Immunohistochemistry with antibodies to melanocyte markers revealed that at the edges of the lesional vitiligo epidermis, melanocytes do not express the kit-protein and the melanocyte-specific microphthalmia transcription factor (MITF-M) [65]. Western blotting confirmed down-regulated expression of c-kit and MITF-M proteins at the edge of the lesional epidermis in vitiligo. These findings strongly suggest a deficiency of the melanocyte survival pathway SCF/c-kit/MITF/Bcl-2 which might be responsible for dysfunction and/or loss of melanocytes in vitiligo epidermis.

Interestingly, we have observed a marked progression of a vitiligo which was stable since many years after treatment with tyrosine kinase inhibitors that inhibit c-kit [66]. Moreover, several cases of vitiligo-like depigmentation occurring after treatment with new tyrosine kinase inhibitors that inhibit c-kit (STI-571 and SU 11428) have been reported [67,68].

The melanocyte growth factor deficient theory

Defective growth and passage capacities of vitiligo melanocytes derived from uninvolved and perilesional skin in vitro have been described [69]. Interestingly, these growth defects of vitiligo melanocytes could be partially corrected in vitro by
the adjunction of fetal lung fibroblast-derived growth factors. In addition, melanocytes taken from actively repigmenting vitiligo macules grow correctly, suggesting a correction of the growth defect. Based on these results, it has been suggested that a decreased concentration of melanocyte growth factor(s) could play a role in the pathogenesis of vitiligo [70].

**Viral infections**

Viral infections have been implicated in the pathogenesis of autoimmune diseases. In one study, CMV DNA was detected in the involved and uninvolved skin of 38% of vitiligo patients and 0% of control subjects. EBV, CMV, Herpes simplex, varicella-zoster, and human T-lymphotropic virus were negative. There are no definitive data to confirm or refute the viral hypothesis. Additional studies are needed to confirm these results [71].

**Melanocyte defective adhesion**

Interaction between melanocytes and the dermoepidermal basement membrane are mediated by integrins (α6β1). Interactions between melanocytes and keratinocytes are mediated by cadherins in association with β-catenin.

Repeated friction in non-lesional skin of vitiligo patients induces detachment and transepidermal elimination of melanocytes [72]. This suggests that minor mechanical trauma in non-lesional vitiligo skin is probably the cause of depigmentation occurring in the Köbner’s phenomenon. Transepidermal elimination of melanocytes in vitiligo may be a possible mechanism of chronic loss of melanocytes, perhaps previously damaged by another process [2].

**Conclusion**

From the available data, it is likely that the loss of epidermal and follicular melanocytes in vitiligo results in melanocyte death. The identification of at least two different clinical phenotypes of vitiligo suggests that melanocyte destruction may be the result of several different pathogenetic mechanisms. Many different hypotheses have been proposed. Recent developments of the genetics of generalized vitiligo strongly suggest a role of immunological factors in generalized vitiligo susceptibility. Besides genetic and immunologic factors, the environment is likely to be involved in the pathogenesis of vitiligo in ways that are not yet known. There are now probably too many hypotheses of vitiligo. All hypotheses are not mutually exclusive. A “consequence” theory suggests that genetic factors, stress, accumulation of toxic compounds, infection, autoimmunity, altered cellular environment, and impaired melanocyte migration and proliferation can all contribute to the phenomenon of vitiligo.

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The basic pathogenesis of vitiligo in general, or for any of the putative subsets of vitiligo, is not fully known. Upon spontaneous or medically induced improvement in vitiligo patches, repigmentation spreads inwards from the borders of lesion. In some color appears diffusely in depigmented areas, but more commonly the pigment spread is perifollicular. The nature of repigmentation can be classified into three types:

1. perifollicular when predominant repigmentation is follicular;
2. marginal when predominant repigmentation is from the borders of patches;
3. diffuse pigmentation when there occurs generalized darkening across the patches of vitiligo.

Melanocytes in human skin reside both in the epidermis and in the matrix and outer root sheath (ORS) of anagen hair follicles. Staricco [1] described two types of melanocytes in hair follicles: the amelanotic or inactive type and melanotic or active melanocytes. Epidermal and amelanotic hair follicle melanocytes proliferated well in culture, whereas the melanotic hair follicle melanocytes did not. Amelanotic hair follicle melanocytes have been shown to differ from epidermal melanocytes in being less differentiated, and expressing less mature melanosome antigens. Nishimura et al. [2] identified the stem cells of melanocyte lineage in the lower permanent portion of mouse hair follicle throughout the hair cycle. They also analyzed the repigmentation process in Tg/+ mice and found that these bulge stem cells are the source of melanocytes in epidermis. Nishimura et al. [3] suggested in another publication that hair graying is due to loss of melanocyte stem cells. Commo et al. [4] also found specific depletion of bulb and ORS melanocytes in graying human hair.

**Mechanisms of medically induced repigmentation**

**Perifollicular repigmentation**

The mechanism of perifollicular repigmentation pattern has been researched extensively and many authors believe that it is the only mode of repigmentation in vitiligo [5]. Repigmentation of vitiligo lesions is thought to occur by the migration of undifferentiated melanocytes from the ORS of the hair follicles to the intrafollicular epidermis. Ortonne et al. [6] demonstrated the mechanism of psoralen plus ultraviolet A (PUVA)-induced repigmentation of vitiligo based on a histochemical and ultrastructural study. They divided the repigmentation process into three stages:

1. proliferation of hypertrophic melanocytes in the lower portion of hair follicle;
2. migration of hypertrophic melanocytes along the hair follicle toward the infundibulum; and
3. migration of melanocytes to the adjacent epidermis.

Similarly Cui et al. [7] have also shown that during repigmentation, treatment stimulated the inactive melanocytes in the middle and/or lower parts of the ORS of hair follicles to divide, proliferate, and migrate upward along the surface of the ORS to the nearby epidermis, where the melanocytes continued to migrate radially to form the pigmented island visible clinically in repigmented vitiligo lesions. It is conceivable that successful repigmentation depends on the availability of migratory factors as well as...