Vitiligo: Medical and Surgical Management
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WILEY Blackwell
To my brother, Sunil who spent his life in the service of the deprived, taught us a lesson in selflessness, and departed this world early.

Somesh Gupta

To my wife Mamie, who has been a consistent source of support and encouragement!

Henry W. Lim

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Mats J. Olsson

To my family and my patients with vitiligo.

Davinder Parsad

To my patients with vitiligo.

Amit G. Pandya

To my inspiring mentor Prof. Jean Marie Naeyaert (deceased), to all of the vitiligo patients, and to my family.

Nanja van Geel
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More than 5000 years ago, human beings were already concerned with white spots appearing on the skin. The impact of such blemishes was so important that in several cultures they were incorporated into their religious beliefs, because they were interpreted as law violations and sometimes sufferers were stigmatized, making vitiligo and other depigmentation disorders very annoying and consequential diseases.

After the discovery of melanocytes by Josue Sangiovanni (1872), the first vitiligo histopathology description by Moritz K. Kaposi (1879) and the finding of intracellular tyrosinase in melanocytes by Bruno Bloch (1929), it was only during the second half of the 20th century and after intensive work, that a compelling amount of information about melanocytes and vitiligo became available, stimulating the search for effective therapies. Subsequently, the availability of topical corticosteroids, psoralens/UVA, NB-UVB phototherapy, topical calcipotriol, together with many other molecules and later calcineurin inhibitors, vitiligo patients achieved excellent, good, or poor treatment outcomes. More recently, a different approach of skin tissue and cellular autografting emerged and provided increased repigmentation rates, becoming important therapeutic options.

Vitiligo, the most important depigmentation ailment, is an autoimmune condition associated with genetic predisposition, oxidative and cellular stress and other factors currently being investigated that may have pathogenic implications. The recent finding on the role of JAK inhibitors tofacitinib and ruxolitinib for vitiligo therapy is a good example of a direct intervention at specific steps in the pathogenic process where immune alterations may be deactivated to prevent pigment cell damage. Another example is the participation of chemokines CXCL9 and CXCL10 in vitiligo, which can be specifically modulated to halt depigmentation. In future years an exponential rise in research to decipher the puzzle of pathogenesis in vitiligo will be possible and a new generation of drugs will emerge.

Vitiligo: Medical and Surgical Management is an excellent book intended to increase awareness of vitiligo as an important and relevant disease that, in spite of being an asymptomatic condition, cripples the self-image of patients and predisposes to social rejection. Vitiligo is a real, acquired disease, not present at birth and not a mere cosmetic alteration for which medical coverage should be provided in every country. In addition, the book is a magnificent resource for physicians interested in treating vitiligo. All chapters, carefully selected by the editors, are written by professors and researchers widely recognized for their work in vitiligo, their innovations and confirmation of their findings.

In the first 20 chapters full descriptions of all basic concepts of the disease are conveyed and illustrated for understanding this condition, and pertinent information on medical therapy for non-segmental and segmental vitiligo is provided. The controversial subject on vitiligo stability is discussed as well.

In the next 30 chapters, surgery is depicted as a very effective solution for the most stable form of the disease, segmental vitiligo, which has the highest repigmentation rates and permanent results. A detailed description of all surgical techniques is presented with emphasis on the appropriate selection of patients, anesthesia, size of affected area, anatomic location, surgical procedures, repigmentation results, and combination therapy, together with results in stable forms of non-segmental lesions, including eyelids, lips, genital, and acral vitiligo. Although less
successful than in segmental vitiligo, the achieved improvement in the latter presentation justifies surgical treatment after failure with medical therapy. Notably, in future trials, permanency of repigmentation for several years, appropriate color blend with normal skin, and patient satisfaction should be defined more clearly as end points for successful results.

Today, more than ever we are getting close to understanding the pathogenesis of vitiligo, and hence are positioned to be able to find safe and potent drugs to stop disease progression and stimulate complete and normal-looking repigmentation. In case of partial or no response, surgical combination therapies can, and should, be added by grafting a few melanocytes where needed. Nevertheless, much work remains to be done to win the fight against vitiligo, a silent but highly disturbing dermatosis.

Rafael Falabella MD
Preface

“...to cure sometimes, to relieve often, to comfort always.”
Ancient folk saying inscribed on the statue of Dr Edward Livingston Trudeau at Saranac Lake, New York.

Why, many would wonder, a whole book on vitiligo, supposedly an incurable autoimmune disease of only cosmetic concerns, with some 53 chapters, written by about as many experts, discussing its management?

Vitiligo is often dismissed as an insignificant cosmetic problem by many healthcare professionals, in spite of the fact that it is associated with significant distress and disruption in quality of life. Until recently, advancement in the treatment of this disease was lacking due to ignorance about its social impact and a lack of interest of physicians and the pharmaceutical industry alike. At present, with the exception of monobenzylether of hydroquinone, a depigmenting agent for removing pigment from residual normally pigmented skin in extensive disease, no other drug has been approved by United States Food and Drug Administration (FDA) for management of vitiligo. However, with the recent improved understanding of the pathogenesis of vitiligo, there is now a renewed interest in the development of new therapeutic options, which provides hope for patients.

Vitiligo is not just skin deep! For many, it is a psychosocial disaster. People with skin of color, in whom the contrast between pigmented and depigmented skin is sharper, have to cope, daily, with the stigma attached to it. Some, who are unable to cope, stop socializing, go into depression, and live an isolated life. This book is aimed at assisting physicians in understanding the disease, curing it sometimes, helping vitiligo patients often, and consoling/counseling them always.

The book discusses all aspect of the disease, from its pathogenesis, classification, psychological aspects, patient support groups, to medical and surgical treatments. A companion video DVD incorporates step-by-step visual demonstrations of common surgical procedures for vitiligo. The editors have invited expert contributors who had performed extensive work within the narrow limits of the chapter they have been assigned. Thus, the reader may expect each chapter to be a masterpiece coming from one of the best experts on that subject. The editors and contributing authors are from Asia, Europe, North America, and Latin America, giving this book a truly global view on the subject.

We started working on this book in early 2014; it took an unusually long time and we editors appreciate the patience of the publisher (John Wiley & Sons) and their editorial staff, individual contributors, and readers who had to wait for three years to see this book become a reality. However, we are sure that the published book will not disappoint them. We also hope that vitiligo patients and treating physicians will benefit immensely from this treatise on the management of a difficult-to-treat disease – vitiligo.

Happy reading!!

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About the Companion Website

This book is accompanied by a companion website:

www.wiley.com/go/Gupta/Vitiligo

The website features:

* Videos
Section 1

Basic Science
Chapter 1
Introduction
Somesh Gupta and Nanja van Geel

A young Indian man with an apparently normal-looking face came to the outpatient department and insisted that he had vitiligo on his face, but which was not visible to the naked eye. When he was examined under a Wood’s lamp, a quite extensive vitiliginous area of the face was present which was not visible to the naked eye (Fig. 1.1). This presentation is not often observed in pigmented Indian skin, and is more common in a caucasian population. On careful examination, the patient had a pale skin which resulted in a loss of contrast between depigmented and normally pigmented skin, which is known to occur in skin prototypes 1 and 2, but not in Indian skin. Vitiligo can surprise even after decades of experience of examining and treating the disease. Not surprisingly, the disease has been described by many as enigmatic.

Vitiligo is Clinically Visible as Cutaneous Depigmentations

Vitiligo is characterized by depigmentations of the skin, mucosa, and hair. Vitiligo can occur/present with different clinical characteristics, prognosis, response to treatment, and course. Broadly, the disease is classified into a localized and a generalized form, or segmental and the non-segmental type. Though in general skin depigmentation occurs much earlier during the course of the disease as compared to hair depigmentation, an exceptional presentation has been described recently, including purely follicular vitiligo, in which only hair follicles are involved and inter-follicular epidermis is spared [1].

Vitiligo is a Disease with an Important Psychosocial Impact

The impact of vitiligo on the quality of life is quite comparable to that of psoriasis, and can be worsened by the social stigma of disease in many cultures [2,3]. Young women in many Asian countries can even suffer more due to gender inequality. Similarly, the disease is more of a concern in people with skin of color due to the increased contrast between depigmented and normally pigmented skin. When evaluating vitiligo treatment, psychological aspects such as quality of life should also be taken into consideration.

Figure 1.2 shows the numbers of articles on psoriasis and vitiligo published since 1980. In contrast to psoriasis, however, vitiligo is not covered by medical insurance in most countries, and currently there is no US-Food and Drug Administration (FDA)-approved repigmentation treatment for vitiligo. This has a negative implication on the interest of the pharmaceutical industry in therapeutic research and drug discoveries in vitiligo.

The present book was aimed at filling this gap, and is intended to place vitiligo in the picture/public eye
Fig. 1.1 (A) An Indian patient insisted that he had vitiligo on the face, though nothing was visible to the naked eye. (B) On Wood’s lamp examination, a segmental vitiligo involving the right upper half of the face became apparent.

Fig. 1.2 Comparison of the number of publications annually in PubMed since 1980, using the keywords ‘Vitiligo’ and ‘Psoriasis,’ respectively.
as a recognized and important disorder. The book is also intended to be a reference for physicians treating patients with vitiligo, and will also demonstrate that vitiligo remains an attractive model to study autoimmunity and understand other diseases, including melanoma.

The Search for New Vitiligo Therapies is on

As skin depigmentations are considered to be asymptomatic, except for sun sensitivity, the prolonged and aggressive use of systemic immunomodulators or systemic steroids is not generally recommended (Fig. 1.3). By using these systemic treatments, lasers and light sources in the spectrum of narrow-band ultraviolet-B, and topical immunomodulators such as calcineurin inhibitors, both an arrest of the disease and a stimulation of melanogenesis can be achieved.

A better understanding of the immunopathogenesis of vitiligo has allowed the identification of many possible targets for biological therapies. Although some biological agents have shown promise, in the absence of large, randomized trials such agents are still not included in the mainstream management strategy for vitiligo. Certain experimental treatments that stimulate melanogenesis, such as afamelanotide and prostaglandin analogs, have appeared promising in early trials. The present book will also include an in-depth discussion on newer approaches for the management of vitiligo.

Because of its sociocultural significance, vitiligo has been a disease of interest for alternative medicine since ancient times. In this book, Chapter 19 is devoted to this topic, while depigmentation therapies (bleaching techniques) are explained in Chapter 51. Such treatment can only be considered in cases of highly extensive therapy-resistant vitiligo.

Surgical Management of Vitiligo

Today, surgical treatment is a recognized component in the management of vitiligo, mainly following the introduction of simplified, cell-based therapies.

When medical therapies stabilize the disease but fail to repigment the skin in areas where melanocyte reservoir in the hair follicles is either lost or absent (such as in glabrous skin), the transplantation of melanocytes can be considered. Non-cultured, cell-based transplantation techniques using melanocyte-enriched cell suspensions are rapidly becoming popular. By using only a small piece of donor skin, larger surface areas can be treated without the need for culture techniques. The same donor area can be used again for cell harvesting after its healing [4], though the main limiting factor for surgical repigmentation in vitiligo is disease activity. Furthermore, even when the major part of the transplanted vitiligo patch becomes repigmented, some depigmented areas may still be left behind (Fig. 1.4). One common disturbing outcome is a peripheral achromatic halo, which has been linked to a possible continued autoimmune activity at the interface between pigmented and depigmented skin.

T helper 17 (Th17) and dendritic cells are known to occur in higher numbers on marginal, actively spreading vitiligo lesions as compared to normally pigmented skin or central depigmented skin [5].
Fig. 1.4 This patient developed an achromic halo after epidermal cellular grafting. Image courtesy of Dr Munish Paul, New Delhi, India.

Fig. 1.5 (A) Even in rapidly spreading early vitiligo with extensive body surface involvement, hair follicle melanocytes can be spared and hairs do not become depigmented until the late stages of the disease. (B) In contrast, in alopecia areata the depigmented hairs can be spared, while only pigmented hair are lost.
The Hair Follicle is Considered an Immunologically Privileged Site

Following identification of the melanocyte stem cell reservoir in the outer-root sheath of the lower part of permanent portion of the hair follicle, some excitement has arisen regarding the use of these cells as a potential therapeutic tool for repigmenting resistant areas of depigmentation [6]. The hair follicle has three distinct populations of melanocytes and their precursor cells: (i) the melanocyte stem cells in the bulge region of the hair follicle; (ii) dopa-negative melanoblasts; and (iii) differentiated dopa-positive melanocytes [7]. Notably, the melanocyte stem cells in the bulge region and intermediate tyrosinase-negative melanoblasts lack all three primary targets of autoimmunity of vitiligo (tyrosinase, gp100, and MART-1) [8], possibly preventing an early depigmentation of the hair during the course of the disease (Fig. 1.5). It remains to be determined whether depigmentation of the hairs is due to exhaustion of the stem cell pool, or to continuous immunological destruction of the melanocytes.

Stability of the Disease and Repigmentation Achieved by Treatment

While different clinical phenotypes are clinically recognized, research groups are currently seeking biomarkers for disease activity. This may be relevant in predicting the prognosis and selecting medical therapeutic modalities as, for instance, in transplantation. However, the question of whether both lesional and global stability are absolute prerequisites for a successful transplantation remains unsolved [9].

In the present book, an attempt has been made to summarize current knowledge on this very important disease. It is hoped that this will be helpful in your practice.

References

The Pigmentary System

Melanocytes are the pigment-producing cells of the human body which provide the skin with its natural color. This offers protection against the hazardous environmental ultraviolet (UV) light. Melanocytes are not exclusively found in the skin but can also be seen in the retina, inner ear, meninges, bones, and the heart. Melanocytes are situated in the basal layer of the epidermis and are surrounded by keratinocytes. By a complex interacting signaling process, they form epidermal melanin units consisting of one melanocyte with 30–40 associated keratinocytes [1]. Melanocytes can be recognized in the epidermis by the expression of tyrosinase (Tyr), tyrosinase-related protein 1 and 2 (TYRP1 and 2), Mart-1, HMB45, and microphthalmia-associated transcription factor (MITF). The produced pigment melanin is packed into specialized lysosome-related organelles called melanosomes, which are transported from the nucleus towards the dendritic tips of the melanocytes. Subsequently, they are transferred by a complex transport machinery to the neighboring keratinocytes. This melanosome transport requires an extensive interplay of numerous factors, which ultimately leads to the distribution of melanin throughout the epidermis, providing a protective shield against UV radiation. Melanogenesis is influenced by several factors including genetics, hormones, UV radiation, and chemical mediators. The variety in human skin color arises by differences in the type of melanin, the rate of melanin synthesis, and the number, distribution, and shape of melanosomes [2,3].

Two main types of melanin exist, namely brown or black eumelanin and red to yellow pheomelanin. The photoprotective capacity of eumelanin exceeds markedly the less efficacious pheomelanin. Pheomelanin is associated with the generation of reactive oxygen species (ROS) after UV radiation [4]. In dark-skin types (phototypes IV–VI), melanosomes contain mainly eumelanin and have an increased size and number compared to lighter-skin types (phototypes I–III). The melanosomes are distributed throughout the whole cytoplasm and are visible in both the basal and upper keratinocyte layers. In lighter-skin types, the melanosomes are retained to an area above the nuclei of the keratinocytes and restricted to the suprabasal layers [5]. This provides darker-skin types with a higher protection against photocarcinogenesis and photoaging, as the UV light reaching the upper dermis is reduced approximately fivefold compared to light-skin types. There is no difference in the density of melanocytes across the different skin types. Several genetic polymorphism have been found that account for differences in skin pigmentation. In the European population, more than 60 single nucleotide polymorphisms (SNPs) in the MC1R gene have been detected which can alter the activity of the receptor, leading to a variety of skin and hair phenotypes [6].
Embryological Development

The precursor cells of melanocytes, which are termed melanoblasts, originate from a subset of multipotent stem cells located at the neural crest. Melanoblasts display expression of MITF, paired box gene 3a (Pax3a), and Sry-related HMB-box (Sox10). MITF is essential for melanoblast survival, and is a key enzyme of melanogenesis by regulating tyrosinase and tyrosinase-related protein 1 (TRP1). PAX3 and SOX10 are also involved in melanoblast survival and differentiation. Combined, they regulate the transcription of MITF [7]. During embryogenesis the melanoblasts migrate from the dorsal side of the neural tube in a ventrolateral fashion, and mature into pigment-producing melanocytes. The embryological migration and differentiation of melanoblasts is regulated by numerous signaling molecules. The Wnt signaling pathway has, besides an important role in melanogenesis and cancer, also a regulatory function in embryogenesis. Wnt proteins are ligands of the frizzled family cell surface receptors. This results in activation of the dishevelled family proteins, which normally inactivates a multiprotein complex containing axin, adenomatous polyposis coli (APC), casein kinase Ia (CKIα), and glycogen synthase kinase-3b (GSK3b). As a result, the degradation of β-catenin is inhibited. This is a crucial event in embryogenesis, as optimal concentrations of β-catenin are required in the development and migration of melanocytes. Other important regulatory factors include endothelin-3 (ET-3), stem cell factor (SCF), c-Kit ligand, bone morphogenetic proteins (BMPs), and hepatocyte-growth factor (HGF). These molecules bind to their corresponding cell membrane receptors inducing a signaling cascade which regulates cell migration and differentiation [8].

Melanogenesis

The synthesis of melanin is initiated by hydroxylation of L-phenylalanine to L-tyrosine, or directly from L-tyrosine. Tyrosinase hydroxylates L-tyrosine, resulting in 3,4-L-dihydroxyphenylalanine (L-DOPA), which further undergoes oxidation to dopaquinone. This makes tyrosinase an essential and rate-limiting enzyme in melanogenesis (see Fig. 2.1). The central role of tyrosinase is illustrated

Fig. 2.1 Melanogenesis and main signaling pathways.
by the effect on pigmentation of inactivated tyrosinase in oculocutaneous albinism. Subsequently, two key downstream pathways diverge that result ultimately in the production of black-brown eumelanin or yellow-red pheomelanin.

In the presence of cysteine, dopaquinone is converted to cysteinyldopa, leading to the production of pheomelanin [9]. In the absence of cysteine, dopaquinone cyclizes to dopachrome and is converted to dihydroxyindole (DHI)-melanin, which has a dark brown to black color. If TRP2 is available, a lighter brown pigment is formed called DHI-carboxylic acid (DHICA)-melanin. The mixture of these three types of melanin give rise to the visible variation in human skin pigmentation [10].

**Melanocortin 1 Receptor (MC1R) Signaling**

The MC1R is a G protein-coupled transmembrane receptor on melanocytes. The MC1R is activated by the binding of melanocortins, a structurally related family of peptides derived from the precursor peptide proopiomelanocortin (POMC) after proteolytic cleavage [11]. The melanocortins involve α-, β-, and γ-melanocyte-stimulating hormone (MSH) and andrenocorticotropic hormone (ACTH). The ACTH molecule has the same first 13 amino acid sequences as MSH, and exerts a similar activity. The activated MC1R binds to Gas proteins that activate adenylate cyclase (AC) [12]; the latter is able to catalyze the conversion of ATP to cAMP. The intracellular accumulation of cyclic AMP (cAMP) is an important factor in the regulation of melanogenesis, and leads to a complex downstream transduction pathway. One of the major events is the phosphorylation of protein kinase A (PKA). Phosphorylated PKA is translocated to the nucleus where it activates cAMP-responsive element binding (CREB) proteins. CREB binds to a CRE domain in the promoter region of the MITF gene. As such, this transcription factor induces the expression of MITF, which is a pivotal regulator of melanogenesis, by regulating the expression of tyrosinase (TYR), tyrosinase-related protein 1 (TRP1), tyrosinase-related protein 2 (TRP2) (= dopachrome tautomerase), PMEL17 and RAB27A [13]. cAMP also improves the binding affinity of MITF to the M-box of the TYR gene [14].

Activation of this pathway leads to the production of eumelanin and the proliferation of melanocytes [15]. The inactivation of MC1R signaling results in pheomelanogenesis, and inactive MC1R variants are linked to the red hair light-skin phenotype and enhanced risks of melanoma [16]. Besides genetics, MC1R expression is influenced by numerous environmental factors, such as UV radiation, endothelin-1, basic fibroblastic growth factor (b-FGF), and proinflammatory cytokines [17].

**KIT Signaling Pathway**

The KIT signaling pathway acts by phosphorylating mitogen-activated protein kinase (MAPK), which upregulates MITF expression. Following the binding of stem cell factor (SCF) to the c-KIT receptor on the melanocyte cell membrane, a dimerization of two subunits is initiated, with subsequent autophosphorylation of tyrosine. The activated c-KIT receptor recruits several adapter proteins, including growth factor receptor-bound protein 2 (GRB2), Src homology 2 domain-containing transforming protein 1 (SHC), son of sevenless (SOS), and SH2 domain-containing protein tyrosinase phosphatase (SHP2). This leads to activation of the Ras-MAPK pathway that ultimately enhances the expression of MITF [18].

**The Melanosome**

Melanosomes are specialized organelles that transfer melanin pigments along a microtubular network from the melanocyte nucleus to the cell periphery and the adjacent keratinocytes. Their development is characterized by four stages (Fig. 2.2). Stage I and II melanosomes are termed premelanosomes; in the early phase these exhibit an electron-lucent image. Melanin synthesis starts from stage 3 melanosomes. The melanosome transport is orchestrated by two major motor proteins: (i) kinesin, which is necessary for the anterograde movement toward the cell periphery; and (ii) dynein, which is involved in the retrograde movement to the cell center. While dynein is mostly expressed in early-stage melanosomes, kinesin is found in mature melanosomes, which corresponds with the delivery of end-stage melanosomes to keratinocytes [19].
At the cell periphery, the melanosomes make contact with the actin network of the cell membrane. This process necessitates a tripartite protein complex containing myosin Va (MYOVA), RAB27A, and melanophilin (MLPH). Mutations in one of the corresponding genes lead to disorders in skin pigmentation, such as the rare autosomal recessive Griscelli syndrome in which a hypopigmented and silvery hair phenotype is displayed [20].

References


Chapter 3

Epidemiology of Vitiligo

Nader Aboul-Fettouh and Amit G. Pandya

Introduction

Vitiligo is the most common depigmenting disorder, with an estimated prevalence ranging from 0.5% to 2.0% of the worldwide population [1,2]. The prevalence of the disease varies by geographical region and age, but not by gender. Epidemiological surveys throughout recent decades highlight the non-Mendelian inheritance pattern of vitiligo and associated autoimmune comorbidities, likely related to the autoimmune pathophysiology of vitiligo. The epidemiology of vitiligo among several populations will be reviewed in this chapter, and the unique distribution patterns of the disease will be summarized.

International Prevalence of Vitiligo

Although worldwide prevalence has been estimated to be 0.5–2.0% of the population, there are large geographic differences [2]. For example, an estimate in the Shaanxi Province of China reported a prevalence as low as 0.093% [3], whereas regions of India had rates as high as 8.8% [1]. However, this high estimate could be due to the inclusion of chemically induced depigmentation, as well as the limited study population and possible sampling bias, as it included only one skin institute in Delhi. One of the largest epidemiological surveys to have been reported was performed on the Isle of Bornholm, Denmark in 1977, and stated that 0.38% of the population was affected [4]. Prevalence has also been reported as high as 4% in some South Asian, Mexican, and US populations [5]. Significant differences in the distribution of vitiligo have been reported within various regions of the world, specifically East Asia. In a study conducted in Japan, a total of 67,448 patients who presented to 190 dermatology clinics were reviewed; the results demonstrated a disease prevalence of 1.68% and a preponderance in elderly people [6]. In contrast, various research groups identified the annual prevalence of vitiligo in Korea to be 0.12%, 0.13%, and 0.13% between 2009 and 2011 [7]. However, the Korean study included patients from the general population who were referred to a primary, secondary, or tertiary referral hospital with an ICD-10 code for vitiligo, rather than those presenting to dermatology clinics. This may have contributed to the stark differences in prevalence. In the Shaanxi Province of China, a research group used a door-to-door survey to identify vitiligo. Suspected vitiligo patients were subsequently verified by a skin examination conducted by dermatologists. Among the 42,833 persons studied, the prevalence was found to be 0.093% [3]. With largely varying methods having been employed among these studies, attempts at estimating the true prevalence of vitiligo in East Asia have been difficult.

The perceived incidence of vitiligo may be higher in populations with darker skin types, as it is more easily detected in skin of color. In an eight-year prevalence study conducted in Greece, where skin types III and
above predominate, vitiligo was found to be more frequently diagnosed in the spring and summer (64.4%) compared to winter and autumn (35.6%) [2], most likely due to the lesions of vitiligo being more easily identify on darker skin. However, no reliable data are available to support this hypothesis.

**Gender Equivalency of Vitiligo**

The male-to-female incidence ratio of vitiligo has long been a matter of debate. Vitiligo is widely considered to have an autoimmune basis [8], and gender equivalency is generally unusual among autoimmune diseases. However, a review of literature showed that vitiligo affects males and females in equal proportions [1,2,4,6,7]. Although some studies have reported a female preponderance [5,9], this discrepancy has been attributed to a variety of reasons. Due to the cosmetic concerns of vitiligo, it is thought that women are more likely to seek treatment at a dermatologic center than are men. Women have also been found to acquire the disease at a younger age than men [10]. In an eight-year prevalence study conducted in Greece, the disease was significantly more prevalent among women aged less than 30 years. The male peak prevalence was noted in the 51–60-year age group, and disease was much more common in middle-aged (31–60 years) than in younger men [2]. It is unclear why women acquire the disease at an earlier age. This may be due to a greater autoimmune predilection in women, though this point remains to be demonstrated in vitiligo specifically. Affected siblings of vitiligo patients also demonstrate an equal gender distribution [9].

**Age of Presentation of Vitiligo**

Vitiligo commonly begins in childhood or young adulthood, with peak onset at 10–30 years of age [5]. Segmental vitiligo occurs before the age of 30 years in 87% of cases, and before an age of 10 years in 41.3% [1]. Although segmental vitiligo tends to occur at a young age, non-segmental vitiligo can affect all ages but rarely presents in infancy or in old age. Females tend to present with vitiligo at an earlier age, with a peak prevalence during the first decade of life, whereas male peak prevalence is in the fifth decade of life, as previously noted [10].

Differing reports have been published on the prevalence of childhood-onset vitiligo. An epidemiological survey conducted on the isle of Bornholm, Denmark showed that almost 50% of people develop vitiligo after the age of 40 years [4], while other reviews have reported that 50% of cases appear before the age of 20 years [5]. Dermatological investigations of school children found that vitiligo prevalence was 0.04% of school pupils in Brazil, 0.17–2.16% in India, and 0.4% in Sweden. The reported prevalence of vitiligo has ranged from 0.05% to 2.28% in the general population, and from 0% to 2.16% among children, which suggests that the worldwide prevalence of childhood and adult vitiligo is not different [1,11].

**Vitiligo and Associated Comorbidities**

One major reason for the autoimmune basis of vitiligo is the disease's association with many other autoimmune conditions. Non-segmental vitiligo has been frequently associated with autoimmune thyroid disease, especially Hashimoto’s thyroiditis [1]. In a survey of 1802 vitiligo probands from North America and the United Kingdom, 19.4% of patients reported a clinical history of autoimmune thyroid disease – an eightfold increase over the 2.39% population frequency of self-reported clinical autoimmune thyroid disease among the same population [9]. Similarly, 16.4% of vitiligo patients in Korea were found to have coexisting thyroiditis [7]. Although thyroid disease is the most common coexisting autoimmune condition associated with vitiligo, pernicious anemia, Addison’s disease, systemic lupus erythematosus, inflammatory bowel disease, atopic dermatitis, insulin-dependent diabetes mellitus, scleroderma, psoriasis, and alopecia areata have also been observed in higher frequencies among vitiligo patients compared to the general population [7,9]. The frequencies of these autoimmune diseases were likewise increased in the probands’ first-degree relatives. No significant increases in the frequencies of multiple sclerosis,
myasthenia gravis, rheumatoid arthritis, or Sjögrens syndrome have been reported.

Inheritance Patterns

Although the inheritance of vitiligo occurs in a non-Mendelian pattern [5], the disease does have a significant genetic component. First-degree relatives of Caucasian and Indo-Pakistani probands displayed 18-fold and 13-fold increased relative risks of the disease, respectively [9]. Concordance of vitiligo was found to be 23% among 22 monozygotic twin-pairs, which was higher than the overall frequency of vitiligo in siblings, while dizygotic twins had a concordance of 0% [9]. Up to 40% of patients with vitiligo noted a positive family history [5]. Interestingly, the mean age of onset has been found to be earlier in those with a positive family history [10]. Patients with a younger age of onset show a greater fraction of affected relatives than those with later-onset vitiligo, suggesting a larger genetic component in early-onset families [9]. Whilst no genes or chromosomal regions have been confirmed as being implicated in vitiligo susceptibility [9], several genes – including HLA, PTPN22, NALP1, and CTLA4, all of which are associated with autoimmune susceptibility – have been association with vitiligo [5].

The limited concordance in monozygotic twins indicates a major non-genetic component in the development of vitiligo which is similarly seen in other autoimmune pathologies. Many patients with vitiligo attribute the onset of their disease to specific life events including, but not limited to, trauma, emotional injury, illness, or pregnancy. Nevertheless, there is no proof that these factors precipitate vitiligo [5]. Further studies are required to characterize the genetic and environmental factors that play a part in vitiligo susceptibility.

Conclusions

With estimates of the prevalence of vitiligo ranging from 0.5% to 2%, the disease is thought to affect several hundred million people worldwide. Whilst the overall incidence of the disease is equivalent in males and females, vitiligo presents with an earlier peak prevalence in females. There is strong evidence for a mixed genetic–environmental influence of vitiligo, although no specific genes have been confirmed as being involved. Patients with vitiligo are likely to have associated autoimmune comorbidities, with thyroid disease (largely Hashimoto's thyroiditis) presenting in about 20% of patients [9].

References


Chapter 4
Pathogenesis of Vitiligo
John E. Harris

Introduction

Vitiligo is an autoimmune disease of the skin that results in depigmented macules and patches due to the destruction of melanocytes. Current treatments for vitiligo have moderate efficacy, but their mechanisms of action are relatively unclear, partially due to their broad, pleiotropic effects on multiple systems and tissues in the body, and partially due to the limited knowledge of vitiligo pathogenesis. However, there has been a recent rapid increase in an understanding of the mechanisms responsible for vitiligo, and with this increase a growing optimism that more targeted and effective therapies with an improved safety profile will soon become available for use in the clinic. The aim of this chapter is to discuss the pathophysiology of vitiligo as it relates to current, emerging, and future treatments.

For decades multiple groups have focused their studies of vitiligo on either the melanocyte and its abnormalities, or the immune system and why it initiates and continues the destruction of melanocytes. In fact, these independent investigations have resulted in the belief that these were mutually exclusive theories of pathogenesis, that either the melanocyte was defective, or that it was the normal target of autoimmunity. Recent studies now tie these two theories together, offering data to support a ‘convergence’ theory (as suggested prior to these studies) that both intrinsic melanocyte abnormalities and autoimmunity synergize in vitiligo pathogenesis [1]. Advances in genetic studies of vitiligo support this convergence theory, and will be discussed in more detail below.

Thus, there are multiple abnormalities in vitiligo that may be targeted for treatment. In addition to the loss of melanocytes in vitiligo, successful treatment requires their regeneration, differentiation, and migration back into lesions of the skin to cause clinically apparent repigmentation. Thus, new, targeted treatments should not only consider how to stabilize or stop the causes of vitiligo (melanocyte defects and autoimmunity), but also how to promote repigmentation of the skin through melanocyte regeneration. This chapter will focus primarily on vitiligo pathogenesis and its influence on medical and surgical management, while the mechanisms of repigmentation will be discussed in more detail in Chapter 5.

Vitiligo Pathogenesis

Melanocyte Abnormalities

Multiple groups have reported that melanocytes isolated from the non-lesional skin of vitiligo patients were not normal when compared to those isolated from healthy controls. They were more difficult to culture in vitro, as they grew slowly and were more susceptible to oxidative stressors, including hydrogen peroxide [2,3]. In addition, they exhibited ultrastructural defects, including a dilated endoplasmic reticulum and oxidized membrane lipids, features
that suggested an elevated level of oxidative stress within the cell [4–6]. These observations were also supported by in vivo studies that revealed elevated levels of reactive oxygen species (ROS) in the epidermis of vitiligo patients, as well as decreased levels of catalase, an enzyme that detoxifies oxygen radicals and relieves oxidative stress [5,7].

Specific commercial chemicals have been shown to both initiate and exacerbate depigmentation in patients with vitiligo, including monobenzyl ether of hydroquinone (monobenzone), 4-tert-butylphenol (4-TBP), and others [8]. Recent studies have revealed that these chemicals act as tyrosine analogs, and also induce oxidative stress in melanocytes, correlating their ability to induce vitiligo in vivo with the induction of oxidative stress within the melanocyte. In addition to inducing ROS, these chemicals activate the unfolded protein response (UPR) and autophagy – both pathways that are intricately involved in the response to cellular stress [9,10]. Thus, melanocytes from vitiligo patients exhibit intrinsic abnormalities that include elevated oxidative stress, and vitiligo-inducing chemicals also induce oxidative and other cellular stress responses, suggesting that these abnormalities are important in the pathogenesis of vitiligo.

Stressed melanocytes have been reported to produce pro-inflammatory signals that activate the local immune response in the skin, which possibly serves to bridge the intrinsic melanocyte abnormalities to activation of autoimmunity that targets them. One group reported that 4-TBP-induced melanocyte stress results in the secretion of interleukin (IL)-6 and IL-8 from melanocytes, potentially promoting inflammation. Others suggest that, rather than producing inflammatory cytokines directly, stressed melanocytes produce danger-associated molecular patterns (DAMPs) that are detected by nearby dendritic cells and serve to activate them to initiate inflammation [11]. One group found that chemical-induced stress in melanocytes results in the secretion of induced heat shock protein 70 (HSP70i), which serves as an immune adjuvant to activate dendritic cells (DCs) in the skin [12]. Another group reported that melanocyte stress initiates autophagy (or ‘self-eating’) of melanosomes within the cell. These degraded intracellular vesicles contain enzymes and products of melanogenesis, which then become incorporated into vesicles that are secreted by melanocytes as exosomes. Exosomes are small, approximately 100 nm vesicles that contain melanocyte antigens (including enzymes of melanogenesis) as well as DAMPs that activate the DCs that take them up. These events serve as a plausible connection between intrinsic abnormalities observed in melanocytes and inflammation responsible for their destruction.

**Innate Immunity**

The results of recent studies have suggested that innate immune activation occurs in the skin of vitiligo patients, and may be responsible for initiating the disease. Natural killer (NK) cells are important components of innate immunity that help to control viral infection as well as tumor growth. They are capable of direct cytotoxicity of target cells, as well as cytokine secretion, including interferon-gamma (IFN-γ). One study reported infiltration of not only lesional but also non-lesional skin of vitiligo patients with NK cells [13], suggesting that they are early initiators of disease, possibly by detecting melanocyte stress. If NK cells become activated in the skin of patients, they may become cytotoxic and directly damage or destroy melanocytes themselves, or instead produce cytokines such as IFN-γ to promote the infiltration of T cells that then drive depigmentation. Further studies will be required to determine the role that NK cells play in the pathogenesis of vitiligo, and whether targeting this population will be an effective treatment strategy.

A second population of innate immune cells implicated in vitiligo pathogenesis is the inflammatory DC, reported both in lesional human skin and blood, as well as in a mouse model of vitiligo. These inflammatory DCs may initially become activated by the melanocyte stress-induced DAMPs discussed above, including HSP70i. When DCs are exposed to HSP70i in vitro, they become activated and mature [12], supporting the hypothesis that they are generated by melanocyte stress-induced HSP70i. When HSP70i was added to the vaccination of mice with melanocyte antigens, depigmentation was accelerated, demonstrating its role as an immune adjuvant in vivo as well [14]. When HSP70i-deficient mice were exposed to a vitiligo induction protocol, they failed to develop robust depigmentation, suggesting its presence is required for the development of vitiligo [15]. Melanocyte-derived exosomes were also found to induce activation of DCs in vitro, and may serve
as another mechanism for the generation of inflammatory DCs. Whether HSP70i is a key DAMP in exosomes is currently unknown. In summary, innate immune cells such as NK cells and inflammatory DCs may sense melanocyte stress and activate inflammation to initiate depigmentation in vitiligo. The cooperation between melanocyte stress and innate immune populations to induce inflammation is summarized in Figure 4.1.

Adaptive Immunity

The primary effector cells of vitiligo are melanocyte-specific cytotoxic CD8+ T cells, which migrate into the skin, find their melanocyte targets, and destroy them. This was initially suggested by the presence of CD8+ T cells infiltrating lesional epidermis and their close proximity to dying melanocytes [16]. Their functional role in driving depigmentation in vitiligo was revealed when lesional skin was obtained from vitiligo patients and the infiltrating T cells isolated and expanded. These cells included both CD4+ and CD8+ T cells, and a large number of the CD8+ T cells were melanocyte-specific and capable of killing melanocytes in vitro. When the mixed T-cell populations were incubated with non-lesional skin from the same patient, they migrated into the skin, found the melanocytes, and induced apoptosis. CD8-depleted T cells were unable to kill the melanocytes, while purified CD8+ T cells were even more potent [17]. These studies revealed that CD8+ T cells are necessary and sufficient to destroy melanocytes in situ.

Lesional CD8+ T cells were found to produce IFN-γ, tumor necrosis factor-alpha (TNF-α), and other cytokines, including some that produced IL-17 [17]. A previous analysis of gene expression in lesional vitiligo skin reported elevated levels of IFN-γ as well [18]. Active lesional skin from patients with vitiligo was found to express an IFN-γ signature that included prominent expression of the IFN-γ-induced chemokines CXCL9, CXCL10, and CXCL11. CXCR3, their common receptor, was also found to be elevated on melanocyte-specific CD8+ T cells in patient blood and skin, and CXCL10 was elevated in vitiligo patient serum [19]. The present author's group and others, using independent mouse models of vitiligo, found that IFN-γ was required for depigmentation [20–22]. Further, it was determined that the IFN-γ-induced chemokine CXCL10 was required for both the progression and maintenance of depigmentation in vitiligo. Indeed, blocking CXCL10 with a neutralizing antibody both prevented and reversed vitiligo, supporting this as a viable strategy for new treatments [19]. The source of IFN-γ required for the initiation of
disease by recruiting the earliest autoreactive CD8⁺ T cells is not clear. NK cells are well-known IFN-γ producers, and are good candidates for this role.

While CD4⁺ T cells infiltrate lesional skin in vitiligo, their role in vitiligo pathogenesis is unclear. They may help to produce IFN-γ to promote the progression of vitiligo, although CD8⁺ T cells can also directly produce IFN-γ. In fact, one group reported that depleting CD4⁺ or CD25⁺ T cells in a mouse model of vitiligo resulted in a worsening of disease, which suggested that these cells primarily prevented or controlled depigmentation [20]. The most likely explanation for this is that T regulatory cells (Tregs), a subset of CD4⁺ T cells that produces CD25, play an important role in controlling inflammation and suppressing autoimmunity. Other groups demonstrated that infusing Tregs into mice induced with vitiligo had reduced disease, further implicating them in the control of vitiligo [22,23]. Multiple studies have suggested that Tregs play a role in human vitiligo, yet how they may be defective in patients with vitiligo is not yet clear, as the results of the studies do not agree [24–28].

**Genetic Factors**

A number of genome-wide association studies (GWAS) have identified risk alleles that influence the development of vitiligo. Genetic risk variants include melanocyte-specific alleles (tyrosinase, melanocortin 1 receptor, and OCA2), stress-associated genes (XBP1), and genes associated with innate immunity (NLRP1, TICAM1, IIFH1, etc.), and adaptive immunity (HLA-A, GZMB, IL2RA, and others) [29], supporting the proposed roles of melanocyte stress, innate immunity, and adaptive immunity discussed above. Interestingly, the risk of vitiligo and the risk of developing melanoma appear to be inversely correlated, such that those patients with vitiligo have a reduced risk of melanoma, and vice-versa [30]. This suggests that vitiligo may result from an over-reactive protective immune response against melanoma. Importantly, the development of treatments for vitiligo should also consider risks associated with promoting melanoma.

**Relevance to Treatment Approach**

When developing an approach to the management of vitiligo, three general aspects of the disease should be considered. First, intrinsic melanocyte abnormalities are present, which may be influenced by inherited genetic risk factors, as well as environmental exposures. Second, autoimmunity plays a key role in driving the progression of vitiligo, and is largely responsible for the loss of melanocytes leading to depigmentation. Both innate and adaptive arms of the immune system are likely to contribute. Third, once the disease is stable – which may occur naturally in segmental vitiligo or through treatment – melanocytes must proliferate, migrate, and differentiate within lesional skin for repigmentation to occur (Fig. 4.2). Any or all of these three components could be targeted with treatments (as well as avoidance of stress-inducing chemicals), and it is likely that the more that are included in treatment strategies, the better.

**Medical Treatment**

The medical treatment of vitiligo currently focuses primarily on the autoimmune component of vitiligo. Current treatments include topical immunosuppressants (steroids and calcineurin inhibitors) and phototherapy, which appears to function at least partially through immunosuppression, although it is also likely to promote melanocyte regeneration as well, which may be why it is so highly effective. Some have suggested that stabilizing melanocyte stress by administering antioxidants either topically or orally may improve current treatments, though they have not yet been proven highly effective. Topical pseudocatalase cream was reported to improve the response to phototherapy [5], but this has not been consistently shown in multiple independent studies [31]. Oral antioxidants such as herbal supplements and vitamins have also been proposed as adjuvants to conventional therapies, but more studies are needed to verify their effectiveness [32].

*Immunosuppressive treatments* are the mainstay of current therapy, and likely function through general immunosuppression, while more targeted therapies may be more effective with an improved
safety profile. As such, many have proposed targeting the immune pathways described above as an approach to develop new targeted treatments. For example, one group found that the exposure of DCs to a mutant form of HSP70i prevents their maturation into pro-inflammatory cells, and therefore this altered protein may prove to serve a therapeutic function if it can be effectively delivered to the correct location in the skin [33]. Another potential option for new medical treatments include targeting IFN-γ or the IFN-γ signaling pathway, which includes the IFN-γ receptor, JAK1, JAK2, STAT1, CXCL10, or its receptor CXCR3 [19] (Fig. 4.3). Multiple small-molecule inhibitors have been developed for JAK1/2 and CXCR3, and neutralizing antibodies have been developed for IFN-γ, CXCL10, and CXCR3. Future studies will be required to determine their effectiveness as treatments for vitiligo.

Fig. 4.2 Treatment goals in vitiligo. Vitiligo pathogenesis consists of intrinsic abnormalities that initiate melanocyte stress and autoimmune destruction of melanocytes. Following successful treatment, melanocytes must proliferate, migrate, and differentiate into lesional skin for repigmentation. This often occurs from melanocyte stem cell reservoirs within hair follicles. Antioxidants such as pseudocatalase and vitamins were proposed as options to stabilize melanocyte stress, although they have not resulted in widespread treatment success. Anti-inflammatory treatments are the mainstay of vitiligo management; however, these act through non-targeted, general immunosuppression. Narrow-band UVB likely acts both to immunosuppress and promote melanocyte regeneration. Surgical treatment promotes repigmentation through the transplantation of melanocytes, but requires stable disease so that autoimmunity does not recur. Emerging and future treatments may target any or all of these aspects of vitiligo.

Fig. 4.3 The IFN-γ pathway is central to vitiligo pathogenesis and may serve as a target for the development of new medical treatments. IFN-γ is expressed in vitiligo lesions and has been reported to be required for vitiligo in multiple mouse models of disease. IFN-γ signals through the IFN-γ receptor, which in turn activates janus kinase (JAK) 1 and 2, as well as STAT1, and induces the production and secretion of CXCL10. CXCL10 signals through CXCR3 to recruit and promote the effector status of autoreactive T cells in vitiligo. Members of this pathway may be targeted for the development of new vitiligo treatments.
Another recent approach to therapy has been the use of afamelanotide as an adjuvant therapy with narrow-band UVB (NB-UVB). As an analog of alpha-melanocyte stimulating hormone (α-MSH), afamelanotide stimulates the regeneration of melanocytes, and has been shown to improve both the speed and extent of repigmentation in vitiligo [34] (see Chapter 18). This is a good example of combination therapy that targets more than one component of disease and results in an improved therapeutic response. Whether afamelanotide is effective on its own, or whether combining its use with other anti-inflammatories will also be effective, is not currently known.

Surgical Treatment

In general, the surgical management of vitiligo through autologous melanocyte or epidermal transplantation is limited to those with highly stable disease. This is due to the fact that, in those patients with active disease, autoimmunity is quickly induced in transplanted skin, which then destroys the transplanted melanocytes. The lack of clear markers of active disease in vitiligo makes the diagnosis of stability difficult, if not impossible, resulting in difficulty selecting appropriate patients for this procedure. However, those with segmental vitiligo progress for only a limited time before depigmentation stabilizes, and therefore typically experience an excellent response.

One possible explanation for this rapid stability and response to surgical treatment is the hypothesis of melanocyte somatic mosaicism in segmental vitiligo. As discussed above, intrinsic abnormalities in melanocytes appear to contribute to the pathogenesis of vitiligo. The *somatic mosaicism hypothesis* is that in segmental vitiligo, only a focal field of melanocytes contains these abnormalities, resulting from a post-zygotic mutation during embryogenesis that then gives rise to a field of abnormal melanocytes in the skin [35–37]. These types of mutation occur quite often, resulting in other skin diseases such as segmental Darier’s disease, where a post-zygotic ATP2A2 mutation in keratinocytes results in a blaschkoid distribution of disease along the affected keratinocyte migration path [38]. If a patient developed such a mutation in a melanocyte that resulted in increased stress, and if the patient also had a predisposition toward autoimmunity, the result might be immune-mediated depigmentation limited to the affected field of melanocytes. In this case, transplanting normal melanocytes from another location to this area could cure the disease, as there would no longer be abnormal melanocytes to trigger depigmentation.

Summary

In summary, vitiligo results from the progressive loss of melanocytes, which leads to the appearance of depigmented macules and patches of the skin. It appears that the disease begins with intrinsic abnormalities within melanocytes that lead to elevated cellular stress, which may be limited to a focal area of the skin in patients with the segmental variant of vitiligo. Innate immune populations may detect melanocyte stress within the skin, and initiate inflammation that promotes the recruitment of melanocyte-specific CD8 T cells that then target melanocytes for destruction. Current therapies focus primarily on suppressing the autoimmune response within the skin by using general immunosuppressive treatments; however, future therapies may be more targeted toward pathways specific for vitiligo, which may result in improved efficacy and safety profiles. In addition, treatments that stabilize melanocyte stress and promote melanocyte regeneration may improve the therapeutic response, and broadening treatment strategies to include all of these components of vitiligo pathogenesis may prove to have the highest efficacy and, in the future, potential for cure.

References


Introduction

Within the skin, melanoblasts reside in the basal layer of the epidermis [1] and in the outer root sheath of hair follicles, where they proliferate and then differentiate into melanin-producing cells, termed melanocytes. The latter are melanin-producing cells with long dendritic processes (dendrites) (Fig. 5.1(A)), with the help of which each melanocyte can make contact with keratinocytes. Melanin is synthesized in specialized intracellular membrane-coated organelles that are present in the cytoplasm of melanocytes, termed melanosomes. The melanosomes, together with melanin, are then transferred to the surrounding keratinocytes with help from the dendrites (Fig. 5.1(B)), where they play an important role in photoprotection against ultraviolet (UV) light.

Melanocytes in human skin reside both in the epidermis and in the matrix and outer root sheath (ORS) of anagen hair follicles. Staricco [2] described two types of melanocytes in hair follicles: (i) amelanotic or inactive melanocytes; and (ii) 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. Recently, Nishimura et al. [3] have identified the stem cells of melanocyte lineage in the lower permanent portion of mouse hair follicle throughout the hair cycle. The same group also analyzed the repigmentation process in Tg/+ mice, and found these bulge stem cells to be the source of melanocytes in the epidermis. In another report, Nishimura and colleagues [4] suggested that hair graying is due to a loss of melanocyte stem cells. Commo et al. [5] also identified a specific depletion of bulb and ORS melanocytes in graying human hair.

Melanoblasts are the stem cells of melanocytes which migrate, proliferate, and differentiate into melanocytes in the skin, and play an important role in repigmentation [6]. Melanocyte differentiation and migration is controlled by various signaling pathway and transcription factors. Several cytokines and growth factors have been implicated in the differentiation of melanoblasts to functional melanocytes. Important genes in this melanocyte developmental pathway include microphthalmia-induced transcription factor (MITF), Paired-box 3 (PAX3), Sex-determining region Y-box 10 (SOX10), endothelin 3 (EDN3), and endothelin receptor B (EDNRB) [7], stem cell factor (SCF), basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), and nerve growth factor (NGF) [8–10].

Keratinocytes in vitiligo lesions release different melanocyte growth factors to stimulate the inactive melanocyte reservoir in the ORS of hair follicles surrounding the vitiliginous patch [11]. The repigmentation of vitiligo lesions occurs by the activation, proliferation, and migration of undifferentiated melanocytes from the outer root sheath of the hair follicles to the intrafollicular epidermis [12–15]. Melanocyte migration involves modifications of the...
architecture of the cells, changes in cell adhesion, and remodeling of the extracellular matrix (ECM). Matrix metalloproteinases (MMPs) are involved in ECM remodeling and in cell migration during a variety of physiologic and pathologic processes. MMPs play central roles in morphogenesis, such as wound healing, tissue repair, migration, and remodeling in response to injury [16,17]. Melanocytes migrating into depigmented vitiligo skin from the ORS in the dermis would need to penetrate the existing ECM tissue barrier. Kumar et al. [18] revealed that the expression and activity of MMP‐2 and MMP‐9 were significantly less in vitiligo patients as compared to controls. The induction of MMP‐2 resulted in significant increases of melanoblast migration on laminin substrates [19]. The expression of MMP-2 activity in supernatants derived from psoralen + UVA (PUVA)-treated melanocytes was significantly increased compared to controls [15]. Wu et al. [20] reported significant increases in MMP-2 and MMP-9 activities in supernatants derived from narrow-band UVB-irradiated melanocytes and keratinocytes.

Repigmentation in vitiligo is initiated by the activation, proliferation, and migration of melanoblasts to the nearby lesional epidermis. The repigmentation patterns were classified as perifollicular, marginal, and diffuse (Fig. 5.2). A perifollicular pattern was evident when the predominant repigmentation was follicular, marginal when the predominant repigmentation was from the borders of patches, and diffuse when a generalized darkening occurred across the patches of vitiligo [21].

**Mechanisms of Repigmentation: Medically Induced**

**Perifollicular Repigmentation**

The mechanism of perifollicular repigmentation pattern has been investigated extensively and many groups consider that it is the only mode of repigmentation in vitiligo [22]. Repigmentation of vitiligo lesions is thought to occur by the migration of undifferentiated melanocytes from the outer root sheath of the hair follicles to the intrafollicular epidermis. Ortonne et al. [23] demonstrated the mechanism of PUVA-induced repigmentation of vitiligo based on a histochemical and ultrastructural study. These authors divided the repigmentation process into three stages: (i) the proliferation of hypertrophic melanocytes in the lower portion of the hair follicle; (ii) the migration of hypertrophic melanocytes along the hair follicle toward the infundibulum; and (iii) the migration of melanocytes to the adjacent epidermis. Similarly, Cui et al. [9] also showed that, during repigmentation, treatment