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Innate immune mechanisms in vitiligo: Danger from within

Jillian M. Richmond, **Michael L. Frisoli**, and **John E. Harris***
Department of Medicine, Division of Dermatology, University of Massachusetts Medical School, 364 Plantation St., LRB 325, Worcester, MA (USA), 01605

Abstract

Vitiligo is an autoimmune disease of the skin in which melanocytes are destroyed by antigen-specific T cells, resulting in patchy depigmentation. While adaptive immunity plays a clear role in disease progression, initiating factors are largely unknown. Many studies report that cellular stress pathways are dysregulated in melanocytes from vitiligo patients, suggesting that melanocyte-intrinsic defects participate in disease pathogenesis. Recent studies reveal that melanocyte stress generates damage-associated molecular patterns that activate innate immunity, thus connecting stress to organ-specific inflammation. Genetic studies in vitiligo support a role for stress, innate immunity, and adaptive mechanisms. Here, we discuss advances in the field that highlight how cellular stress, endogenous danger signals, and innate immune activation promote the onset of vitiligo.

Introduction

The white, patchy depigmentation that is characteristic of vitiligo is disfiguring (Fig 1), and is often psychologically devastating for patients [1]. Approximately 0.5–2% of the world population is afflicted [2]. Factors involved in the initiation of vitiligo are unknown, although both genetic and environmental factors have been implicated [3]. Due to its location within the skin, vitiligo provides an opportunity to directly observe the course of disease, isolate the target tissue for research studies, and culture primary melanocytes, the target cells. Therefore, vitiligo is an excellent disease in which to use translational research strategies to study the pathogenesis of organ-specific autoimmunity. Research into the pathogenesis of human vitiligo over the past 30 years has sparked controversy, as evidence for both autoimmune-mediated destruction of melanocytes and melanocyte-intrinsic abnormalities appeared to be at odds [4]. However recent advances in the field support both hypotheses, and evidence suggests that each is linked to the other through innate immune mechanisms. Several damage-associated molecular patterns (DAMPs) have been associated with cellular stress, and act as ligands for innate pattern recognition receptors (PRRs). It is likely that in vitiligo, stressed melanocytes activate the innate immune system through the generation and release of DAMPs, which provide the initiating danger signal. The inflammation that ensues ultimately leads to activation of the adaptive immune system, thereby facilitating autoimmune destruction and vitiligo progression.

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*Corresponding author: Tel: 1-508-856-1982, Fax: 1-508-856-5463, john.harris@umassmed.edu.

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Adaptive immunity in vitiligo

Multiple studies implicate antigen-specific, $CD8^+$ T cell-mediated destruction of melanocytes in human vitiligo. Early observations reported infiltration of T cells in lesional skin from patients with vitiligo [5], and $CD8^+$ T cells were found adjacent to dying melanocytes in the epidermis [6]. Further, the frequency of melanocyte antigen tetramer-positive $CD8^+$ T cells in the blood of vitiligo patients correlates with disease severity, and these cells are capable of killing melanocytes *in vitro* [7,8]. Finally, purified $CD8^+$ T cells isolated from lesional skin of vitiligo patients, but not CD8-depleted T cells, infiltrate unaffected skin from the patients *ex vivo* and induce melanocyte apoptosis *in situ*, revealing that $CD8^+$ T cells are both necessary and sufficient for melanocyte destruction in human vitiligo [9]. Antigenic proteins in vitiligo have been identified, and include gp100, MART1, tyrosinase, and tyrosinase related proteins 1 and 2 [10–12,7]. Certain mouse models of vitiligo also implicate $CD8^+$ T cells as key effectors in disease, and further identify IFN-γ as a critical cytokine in pathogenesis [13,14]. While $CD4^+$ T cells are present in vitiligo lesions [6], a convincing role for them in pathogenesis has not yet been identified. They are dispensable in a mouse model of vitiligo, and disease is exacerbated in their absence, suggesting a possible role for T regulatory (Treg) suppression [13]. However other mouse models of vitiligo have been developed that are CD4-dependent [15,16]. Tregs are reported to be dysregulated in patients with vitiligo, although there is no clear consensus on specific abnormalities (i.e. decreased numbers, skin homing defects, or functional defects) [17–20].

Cellular stress in melanocytes

The earliest indication that melanocytes from vitiligo patients were intrinsically abnormal was the observation that they were more difficult to culture *ex vivo* compared to those from healthy controls [21], and were more sensitive when exposed to exogenous stressors [22,23]. Ultrastructural analysis revealed a dilated endoplasmic reticulum suggesting increased cellular stress [24], and elevated levels of H_2O_2 and oxidative byproducts reflected oxidative stress [25–27]. Further evidence for the role of stress in vitiligo came from studies on monobenzone and other phenols, commonly found in commercial products (including rubber, leather products, cosmetic dyes, etc.), that are well known to both induce and exacerbate vitiligo [28–30]. While very high doses of these chemicals induced melanocyte death *in vitro*, lower and more likely physiologic doses did not kill the cells but induced reactive oxygen species (ROS) and activated the unfolded protein response (UPR) [31,32]. This effect was dependent on tyrosinase, and occurs through the ability of certain phenols to mimic the chemical structure of the amino acid tyrosine (also a phenol), which is a basic building block of the pigment molecule melanin [31].

In addition to the very specific effect of phenols in melanocytes through interaction with tyrosinase, cellular stress results from oxygen and/or nutrient imbalances, chemical or physical agents, infection, inflammation, and misfolded proteins. Melanocytes are particularly susceptible to stress because they perform melanogenesis, an energy-expensive process by which they produce a large amount of the pigment melanin. Mitochondrial energy metabolism generates ROS, and the production of large quantities of protein increases the risk of protein misfolding in the endoplasmic reticulum, a trigger that activates the UPR. Furthermore, the skin is readily exposed to environmental insults such as UV light, which generates intracellular ROS, hydrogen peroxide, and superoxide anions. Finally, the process of melanogenesis itself liberates hydrogen peroxide, a ROS precursor [33].

Therefore, these data indicate that melanocytes in vitiligo patients are more susceptible to oxidative damage than melanocytes from unaffected individuals due to an inherited inability to manage stressors from normal cellular processes, or exposure to environmental chemicals.

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However while cellular stress in melanocytes provides a reasonable explanation for how vitiligo is induced, it cannot completely account for the disease, since stressed melanocytes remain viable. Bridging the gap between cellular stress and adaptive immunity in vitiligo requires a better understanding of how stress signals are communicated, recognized, and translated into proinflammatory signals. Studies of gene expression profiles in a chicken model of spontaneous vitiligo revealed links between oxidative stress and innate immune activation [34], supporting a role for innate immunity as an important connection between melanocyte stress and adaptive immunity in vitiligo.

DAMPs and other endogenous proinflammatory signals – Danger from within

In contrast to the antigen specificity of adaptive immunity, the innate immune system must rapidly initiate responses without specific antigen recognition, and does so through activation of pattern recognition receptors (PRRs) by “danger signals”. Examples of PRRs include toll-like receptors (TLRs), nucleotide oligomerization domain (NOD)-like receptors (NLRs), and RIG-I-like receptors (RLRs). Many microbe-derived ligands for TLRs have been identified, including lipopolysaccharide (LPS) for TLR4, single-stranded viral RNA for TLR7, and unmethylated CpG bacterial DNA for TLR9. Like TLRs, NLRs and RLRs respond to a variety of ligands including bacterial flagellin and viral nucleic acids. NLRs form large multiprotein complexes known as inflammasomes, which recruit caspases through oligomerization leading to subsequent activation of caspase proteolytic activities. Recruited caspases process proinflammatory cytokine precursors into mature forms that are subsequently secreted – examples include IL-1β and IL-18 [35]. Because PRR ligands are derived from viral and bacterial pathogens, they are referred to as pathogen-associated molecular patterns (PAMPs).

More recently, molecular patterns released during sterile inflammation have been identified that activate PRRs [36]. During sterile inflammation, these patterns are not associated with pathogens but are self-derived following cellular damage, and are thus referred to as damage-associated molecular patterns (DAMPs). For example, in addition to pathogen-derived DNA, NLRP3 inflammasomes are activated in response to ROS [37] and mitochondrial stress [38]. Heat shock proteins (HSPs) are protein-folding chaperones induced in response to cellular stress and UPR activation to improve protein folding, and they activate TLR2, TLR4 and other PRRs [36]. HSP70i is induced following phenol-induced stress in melanocytes and in vitiligo patient skin. In mouse models of vitiligo, HSP70i is required for vitiligo induction [39], accelerates disease progression [40], and activates dendritic cells (DCs) in the skin [41]. Thus, ROS and HSPs generated by melanocytes in response to stress serve as DAMPs in vitiligo, activating PRRs to initiate inflammation.

DAMPs may be secreted from cells, liberated during cellular damage or death, or transported by exosomes. Exosomes are small microvessicles secreted from cells as a means of cell-cell communication [42]. Monobenzone increases exosome secretion by melanocytes, and exosomes deliver known vitiligo target antigens to DCs, contribute to their activation, and lead to the induction of autoimmune T cell responses against melanocytes and melanomas [31]. In addition to antigens, exosomes carry micro-RNAs (miRNAs), HSPs, and other proteins that act as DAMPs. Specifically, the miRNA miR-29b is induced by oxidative stress, and exosomes containing miR-29b induces activation in a macrophage cell line [43]. Tumor-derived $HSP70^{+}$ exosomes stimulate both the activation and migration of human natural killer (NK) cells *in vitro* [44]. Therefore, exosome secretion may provide a means by which melanocytes communicate stress to the innate immune system.

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Phenols that are known to induce and exacerbate vitiligo also activate the unfolded protein response (UPR) in melanocytes, resulting in induction of the transcription factor X-box-binding protein 1 (XBP1) and splicing to its activated form (XBP1s). This leads to production of IL-6 and IL-8, providing a direct link between cellular stress and immune activation [32]. IL-6 antagonizes Treg responses, and IL-6 and IL-8 both promote recruitment of immune cell populations.

Innate cell populations in vitiligo

If melanocyte stress generates DAMPs that activate PRRs and the innate immune response, one would expect evidence of increased recruitment and/or activation of innate immune cell populations in vitiligo. Macrophages, NK cells, and inflammatory dendritic cells (DCs) all infiltrate active vitiligo lesions [6,41,45], however despite the fact that IL-8 is produced by stressed melanocytes and is a chemoattractant for neutrophils, neutrophil infiltration is not characteristic of vitiligo. While the role of macrophages in vitiligo has not been further examined, evidence exists for functional roles of NK cells and inflammatory DCs.

Characterization of the transcriptome of skin from vitiligo patients revealed an innate immune signature reflecting an infiltration of NK cells in both lesional and non-lesional skin. Gene expression correlated with immunofluorescence staining patterns, as NK cells were increased almost five-fold in lesional skin of vitiligo patients when compared to healthy controls, and almost two-fold in unaffected skin. This indicates that patients have a propensity for innate immune activation in the skin, which could tip the balance towards development of autoimmune inflammation, and that NK cells may be important players in vitiligo pathogenesis [45] by directly responding to intracellular stress ligands [46].

Inflammatory DCs, defined as CD11c+ CD11b+, are at increased frequencies in both the skin and peripheral blood of vitiligo patients [41]. These cells are also present in the skin of mice with vitiligo, are induced *in vitro* by the stress-induced protein HSP70i, and are present at even higher numbers in mice that overexpress HSP70i. These studies implicate HSP70i as a crucial signal bridging melanocyte stress and vitiligo induction through innate immune cell activation.

Genome-wide association studies identify potential stress, innate, and adaptive risk alleles in vitiligo

In support of these observations, recent genome-wide association studies (GWAS) reveal multiple genetic risk factors for vitiligo that intersect with these pathways [47]. First, both class I and class II HLA molecules (T cell antigen recognition), PTPN22 (T cell signaling), CD80 (T cell activation), IL2Rα (T cell activation/regulation), GZMB (T cell cytotoxicity), FoxP3 and BACH2 (Treg development and function [48]) implicate a role for adaptive immunity in vitiligo. Next, association of XBP1 with vitiligo supports a role for the UPR pathway in pathogenesis, although it also plays a role in antigen processing. Others found that polymorphisms in XBP1 correlate with the risk of developing Crohn's disease, and its function is through activation of the UPR in a mouse model of inflammatory bowel disease [49]. Finally, IFIH1 (also called MDA5) and NLRP1 are both PRRs that activate innate immune responses, TICAM1 (also called TRIF) is an adaptor protein in TLR signaling, and caspase 7 is activated by inflammasomes [50], all of which implicate an important role for innate immunity in vitiligo. Future studies should focus on the interface between melanocyte stress, innate and adaptive immunity.

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Conclusions and implications – a working hypothesis

In conclusion, both environmental and cell-intrinsic factors trigger melanocyte stress, which results in the production of DAMPs that activate innate immunity, followed by activation of adaptive immune cells, including autoreactive CD8+ T cells that kill melanocytes. Normal melanogenesis, which begins with the amino acid tyrosine as a substrate, generates stress that may be further exacerbated by environmental insults, including phenols and UV light. Melanocyte stress results in production of ROS, activation of the UPR, and release of exosomes. Stress-induced DAMPs, such as HSP70i, serve as ligands for PRRs and activate innate immune cells. Induction of the UPR also results in the direct release of proinflammatory cytokines from melanocytes. Inflammasomes respond to stress signals as well, and while inflammasome activity has not yet been reported within melanocytes, it has been detected in other non-myeloid derived cells. These signals deliver melanocyte-specific antigens and activate PRRs, leading to DC activation and subsequent T-cell priming for melanocyte-specific cytotoxicity (summarized in Fig 2).

These mechanisms may have evolved as a defense against melanoma and malignancies in general, as tumor cells exhibit increased cellular stress. In support of this hypothesis, the risk of melanoma is inversely proportional to that of vitiligo [51]. The precise threshold of acceptable stress before a cytotoxic response is generated still needs to be determined, and may answer how the proper balance between self-tolerance and tumor surveillance is maintained. Cellular stress pathways may also help determine which specific tissue will be targeted in a patient with autoimmunity. Cellular stress has been implicated in other organ-specific autoimmune diseases, including type I diabetes [52] and Crohn's disease [49], implying that mechanisms involved in translating stress to adaptive immunity in vitiligo may be shared. Future studies will be required to identify how intrinsic and extrinsic triggers of cellular stress, innate signaling pathways, and adaptive immune responses work together to initiate, propagate and maintain autoimmunity in vitiligo.

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Highlights

- Melanocytes from vitiligo patients display features of elevated cellular stress
- Stressed cells produce damage-associated molecular patterns (DAMPs)
- DAMPs activate innate immune cell populations through pattern recognition receptors
- Activated innate immune cells can promote adaptive immunity in vitiligo
- Activated stress pathways appear to play a role in organ-specific autoimmunity

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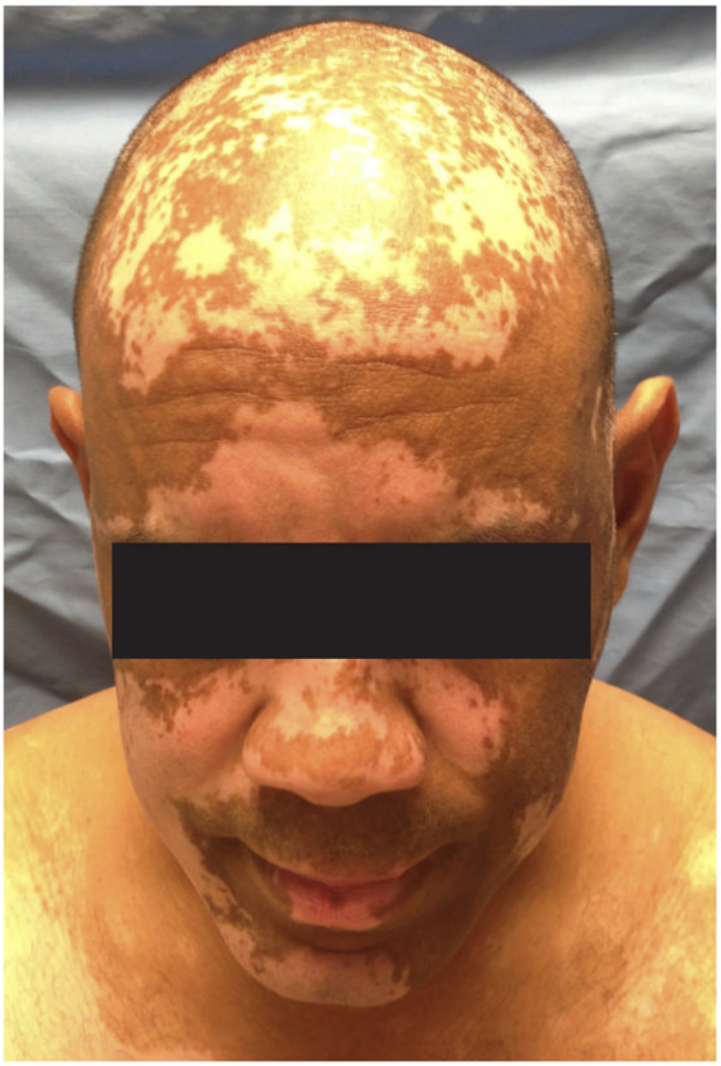

Figure 1.
Vitiligo is characterized by disfiguring white patches on the skin due to the loss of melanocytes.

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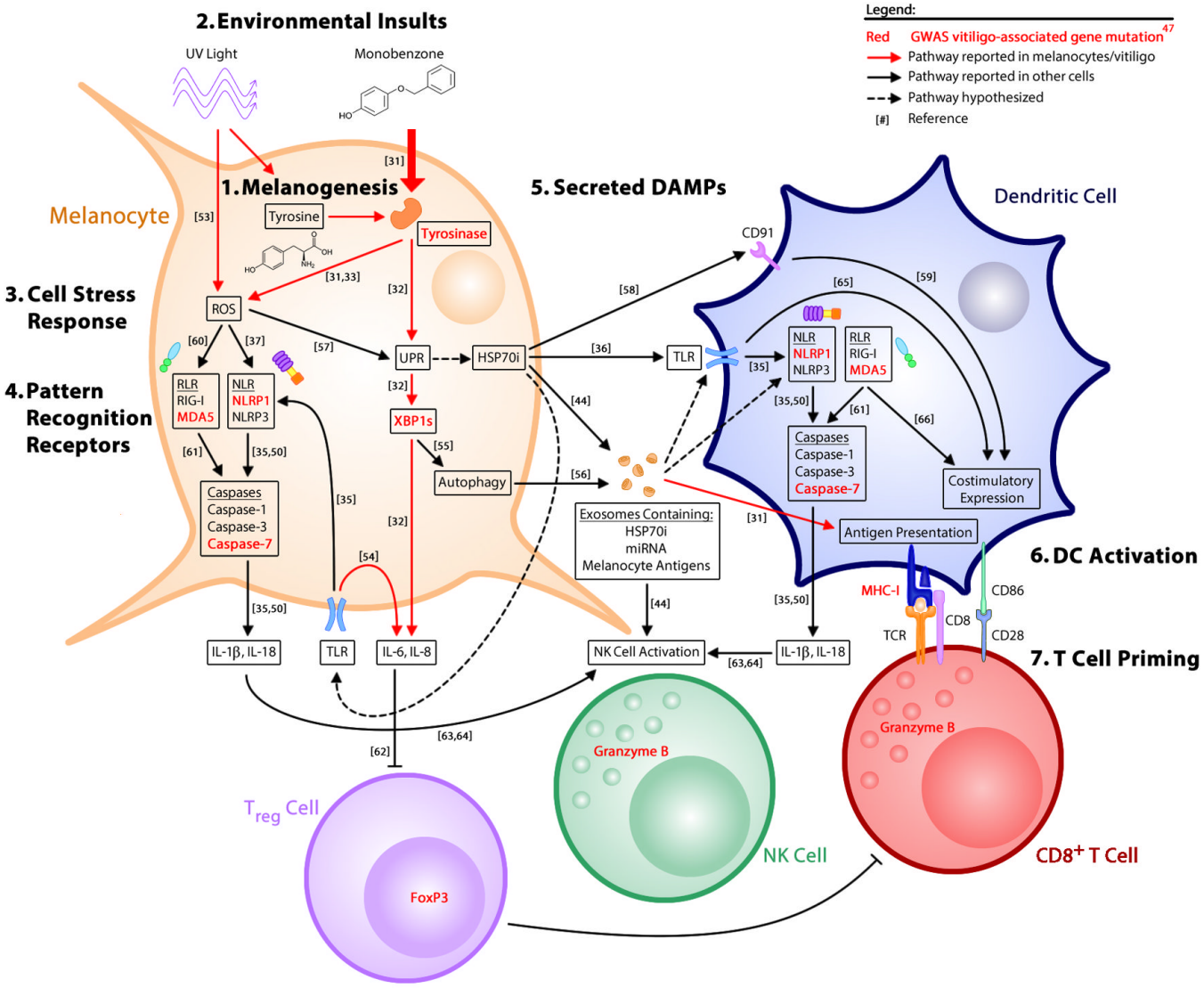

Figure 2. Innate signaling pathways activated by cellular stress lead to adaptive autoimmune responses against melanocytes

The production of melanin results in cellular stress [1]. Environmental insults, including UV light and chemical phenols such as monobenzone, exacerbate this response [2]. Melanocyte stress is characterized by intracellular ROS and activation of the UPR [3], which are both capable of activating PRRs [4] either directly, or through the production of HSP70i and antigen-containing exosomes [5]. These signals function as DAMPs to activate dendritic cells [6] and subsequent priming of $CD8^+$ T-cells for autoimmune attack of melanocytes [7]. Stressed melanocytes secrete low levels of IL-6 and IL-8, which may recruit immune populations and/or antagonize the suppressor function of regulatory T cells (Treg). Abbreviations: DAMP – damage-associated molecular pattern, DC – dendritic cell, GWAS – genome-wide association study, HSP – heat shock protein, NLR – NOD-like receptor, PRR – pattern recognition receptor, RLR – RIG-I-like receptor, ROS – reactive oxygen species, TLR – toll-like receptor, UPR – unfolded protein response. Numbers in brackets correspond to references.