Symposium - Vitiligo

Melanocytorrhagy and apoptosis in vitiligo: Connecting jigsaw pieces

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ABSTRACT

Vitiligo is an acquired depigmenting disorder characterized by a chronic and progressive loss of melanocytes from the epidermis and follicular reservoir. The mechanism of melanocyte disappearance has never been clearly understood. This review discussed the data supporting the theory of melanocytorrhagy and apoptosis as one of the primary defects underlying melanocyte loss. Theory of melanocytorrhagy proposes that non-segmental vitiligo is a primary melanocytorrhagic disorder with altered melanocyte responses to friction and possibly other types of stress, inducing their detachment and subsequent transepidermal loss. Melanocytes detachment induces apoptosis whereas adherence to basement membrane suppresses apoptosis. The study of apoptosis, mechanisms of its induction, and the ways to block apoptosis is one possible way to find both the causes of depigmentation and medications to prevent its progression.

Key words: Apoptosis, melanocytorrhagy, pathogenesis, vitiligo

INTRODUCTION

Vitiligo is an acquired pigmentary disorder of the skin and mucous membranes, and it is characterized by circumscribed depigmented macules and patches. Vitiligo is a progressive disorder in which the melanocytes in the affected skin are selectively destroyed. The prevalence of vitiligo is 0.1 to 3% in different ethnic and racial groups. Because of its pigmentary disfigurement, vitiligo is more significant in the dark-skinned population, with a major impact on the quality of life of patients.[1] It produces social stigmatization and is often confused with leprosy or other socially terrifying infectious diseases. Vitiligo is a multifactorial polygenic disorder with a complex pathogenesis. It is related to both genetic and nongenetic factors. Several theories have been proposed about the pathogenesis of vitiligo, but the precise cause behind melanocyte destruction remains unknown. Theories regarding the destruction of melanocytes include autoimmune mechanisms, cytotoxic mechanisms, an intrinsic defect of melanocytes, oxidant-antioxidant mechanisms, and neural mechanisms.

Autoimmune theory of vitiligo proposes that the melanocytes are killed by autoimmune effector mechanisms, either by memory cytotoxic T cells or by autoantibodies directed against the melanocyte surface antigens. According to neural theory, melanocyte death in non-segmental vitiligo (NSV) is caused directly or indirectly by an inappropriate reaction of the neural-crest-derived pigment cells to neuropeptides, catecholamines or their metabolites, or more generally to an overactive sympathetic system.[2] The impaired redox status theory proposes that NSV melanocyte death results from an intrinsic increased sensitivity to oxidative stress arising either from toxic intermediates of melanin precursors or from other sources (e.g., catecholamines). The vacuolation and degenerative changes noted in NSV skin could be the expression of oxidative damage.[3,4] Low catalase activity leading to epidermal accumulation of H_2O_2 has been demonstrated in non-lesional and in lesional NSV skin

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and in cultured melanocytes.[5] Accumulation of H_2O_2 in NSV skin *in vivo* has been shown.[6] All these theories do not require the death of melanocytes to explain the depigmentation of NSV patches, but speculate either a primary effect on the inhibition of melanogenesis, or on the disappearance of melanocytes because of defective adhesion. It has been already suggested that defective adhesion is involved in melanocyte loss in NSV.[7] The detachment of living melanocytes after continuous friction of perilesional NSV skin has been observed recently. Melanocyte detachment from the basal layer was followed by transepidermal migration and eventually the death of pigment cells.[8] Based on all these theories and findings, a new theory was proposed in this disease. According to this theory, depigmentation in vitiligo patches results from a chronic detachment of melanocytes called melanocytorrhagy, which is possibly related to increased susceptibility to mechanical and other types of stresses like chemical stress.

MELANOCYTORRHAGY IN VITILIGO MELANOCYTES

New theory of melanocytorrhagy proposes that NSV is a primary melanocytorrhagic disorder with altered melanocyte responses to friction and possibly other types of stress, inducing their detachment and subsequent transepidermal loss. Detachment and transepidermal elimination of melanocytes following minor trauma are probably the cause of depigmentation occurring in the isomorphic response known as Koebner phenomenon. It could be speculated that the Koebner phenomenon appears only when melanocyte loss reaches a certain threshold value, variable for each patient. Loss of melanocytes is certainly not balanced by an influx of melanocytes from the follicular reservoir, where melanocytic stem cells are probably situated.[9] Gauthier *et al.*[10] summarized all the theories for NSV, and formulated a new integrated theory which takes into account melanocyte detachment and transepidermal elimination, neural-biochemical, and autoimmune hypotheses.[10] Cario-Andre *et al.*[11] in an *in vitro* study showed that NSV melanocytes have an intrinsic defect, which limits their adhesion in a reconstructed epidermis, with an enhancer effect of the vitiligo keratinocyte milieu.[11] Namazi[12] hypothesized that a combination of pathogenic mechanisms (neurogenic dysregulation, oxidative stress, autoimmunity, and melanocytorrhagy) that act in concert can lead to NSV [Figure 1].[12]

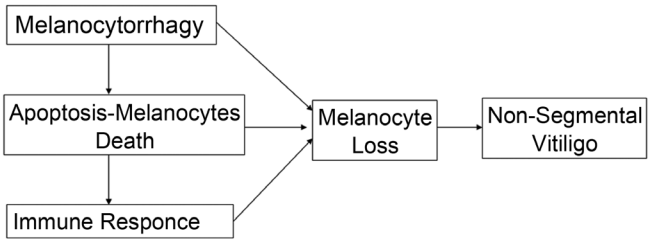

Figure 1: Depicting interconnections between melanocytorrhagy, apoptosis, and immune response, leading to melanocyte loss in non-segmental vitiligo

The baseline expression of adhesion molecules may differ in vitiligo patients as compared to controls. The melanocytes are poorly attached to the basement membrane as compared to keratinocytes. Melanocytes adhesion, spreading, and migration are mediated by integrins, whereas the interactions between melanocytes and keratinocytes are mediated by cadherins.[13,14] Adhesion of melanocytes is also affected by the levels of endothelin-1.[15] *In vitro* melanocyte attachment to laminin is mediated primarily by α6β1 integrins. E-cadherin is the major mediator of human melanocyte adhesion to keratinocytes *in vitro*. Melanocytes dendrites have a very crucial role in the adhesion, migration, and melanosome transfer. In human skin, one melanocyte makes contact with several keratinocytes with the help of dendrites. Integrins that help in dendrite formation are located preferentially along or at the tip of dendrites, whilst integrins that mediate attachment tend to localize over the cell body as well as along the dendrites.[14] The dendritic morphology of melanocytes might depend also on E cadherin homophilic binding.[13] Recently, we have shown that in unstable vitiligo patients, melanocytes were poorly attached to Type IV collagen, whereas stable vitiligo melanocytes and control melanocytes were firmly adhered to Type IV collagen.[16] More importantly, our results demonstrate that dendrites of perilesional unstable vitiligo patients were small with clubbed ends. Dendrite of these melanocytes seems to be retracted, whereas the dendrites of control and stable vitiligo patients were normal in shape and size. Moreover, dendrites increase dramatically the adhesion and anchoring of melanocytes within the basal layer of the epidermis. Routine observation of melanocyte cultures indicates that dendrite retraction comes first, before cell detachment and eventual death. We found no significant difference in the number of dendrites per cell between control, stable, and unstable vitiligo patient melanocytes cultures (Unpublished data). An abnormal morphology of cultured NSV melanocytes

showing stubby dendrites was mentioned by Jimbow *et al*.[17] Loss of dendricity and melanocyte detachment can be induced in melanocyte cultures by the addition of H_2O_2.[18] In normal skin, detachment of melanocytes from the basement membrane and their upward migration in the upper layers of the epidermis has been reported in two non-physiological circumstances, chemical stress[19] and tape stripping.[20] After detachment, melanocytes are seen moving towards stratum corneum. When located in the upper epidermal strata, DOPA- and HMB45-positive melanocytes are no longer dendritic, but become rounded. During their epidermal migration, melanocytes are still incontinent, since melanosomes can be detected outside or within keratinocytes of the upper epidermal layers. Apoptosis has been reported in melanocytes as a function of attachment substrate and growth factors. Human melanocytes adherence to fibronectin suppresses apoptosis, and apoptosis was induced when melanocyte attachment to matrix was inhibited.[21] Early apoptosis of melanocytes cannot be detected in sections when they are in a suprabasal position. Interestingly, a photomicrograph showing a damaged melanocyte located close to the granular layer has been already detected in non-lesional NSV skin.[22]

We demonstrated in our previous study that expression of liver X receptor-alpha (LXRα) at both mRNA and protein level was significantly higher in perilesional skin as compared to the normal skin of vitiligo patient.[23] On treating control melanocytes with LXRα agonist 22-hydroxy cholesterol, the adhesion of melanocytes to type IV collagen and laminin5 decreases significantly. Increase in LXRα expression might decrease the cell adhesion molecule, which ultimately leads to the detachment of melanocytes from the basement membrane in perilesional vitiligo skin which can ultimately lead to melanocytorrhagy (Unpublished data). As such, there are no clinical molecules which can take care of melanocytorrhagy, but work needs to be done on new molecules like molecule targeting LXRα.

APOPTOSIS IN VITILIGO MELANOCYTES

The actual mechanism by which melanocytes are destroyed in the skin of patients with vitiligo has not been definitively determined. There are two known mechanisms leading to the loss of melanocytes. One is the destruction of the cells from extrinsic cytotoxic factors, and is called necrosis. Necrosis is manifested by an infiltrate of inflammatory cells, attacking cells or a tissue. The cells are shattered, and the debris gathered up by macrophages to initiate an immune reaction, either humoral or cellular. The mechanism is especially useful in removing harmful or foreign cells, bacteria, or other microorganisms. The other mechanism by which cells are eliminated is apoptosis, or programmed cell death. There are many histological and ultrastructural features of apoptotic cells. The histological data, and some laboratory data, support apoptosis, rather than necrosis, as the mechanism for removal of melanocytes.

Apoptosis can be induced by a variety of factors, including immune cytokines, some environmental chemicals (for example, substituted hydroquinones such as monobenzone), or other molecular mechanisms. The outcome of a death signal depends on the balance between positive and negative apoptotic regulators, such as members of the Bcl-2 protein family.[24] These proteins consist of an antiapoptotic subfamily, including for example Bcl-2 and Bcl-xL, and a pro-apoptotic subfamily, including for example Bax, Bak, Bid, and Bad.[25] The Bcl-2 family proteins contain both membrane-bound and cytosolic proteins, of which especially the pro-apoptotic members have been described to translocate between subcellular localities during apoptosis.[26] Bax is localized to the cytosol and is mainly bound in a complex with inhibitory proteins.[27,28] In response to an apoptotic stimulus, such as UV irradiation, the interactions between Bax and inhibitory proteins are broken and Bax undergoes conformational changes, enabling the protein to target and insert into mitochondrial membranes.[27-30] This causes mitochondrial release of cytochrome c, which binds to cytosolic Apaf-1, promoting activation of pro-caspase-9 and subsequently pro-caspase-3.[31] Caspase-3 is a key mediator of apoptosis and accelerates a cascade of caspases, leading to degradation of the cell.

Apoptosis in vitiligo is also supported by histology. This is most evident from the changes at the border between depigmented and normal skin. There is damage to the melanocytes and keratinocytes at the borders of the depigmented skin, and a mild mononuclear infiltrate is visible.[32,33] In perilesional skin, DOPA+ cells are larger and highly dendritic, compared to normal melanocytes. In addition, ultrastructurally, the melanocytes exhibited abnormalities such as nuclear shrinkage, vacuolization, and loss of dendrites and detachment.[34] During apoptosis, particles of the dying

cells must be removed by macrophages to eliminate debris and to avoid an immune reaction. The skin might present a special situation. The keratinocytes are avidly phagocytic.[35,36] It is possible that the keratinocytes are able to function by phagocytosing fragmented melanocytes, and carry the debris up toward the stratum corneum and desquamate off. The involvement of keratinocytes in the pathophysiology of depigmentation is consistent with the damage to the melanocytes and keratinocytes seen on histology.

Many environmental chemicals, mainly aromatic or aliphatic derivatives of phenols and catechols like hydroquinone, monobenzyl ether of hydroquinone, 2,4-di-tert-butylphenol, p-tert-butylphenol, p-methyl-catechol, p-isopropylcatechol, p-chlororesorcinol, p-cresol, diisopropyl fluorophosphate, and physostigmine, have been shown to be preferentially toxic to melanocytes, both *in vitro* and *in vivo*.[37-40] These compounds are not toxic to all individuals, but only a subset of human skin depigments in response to application of these compounds.[38] It means these compounds are toxic only to the genetically susceptible. These phenol and catechol compounds are structurally similar to tyrosine, the substrate for tyrosinase that initiates the biochemical pathway for melanin synthesis.[40] Because of this similarity, these compounds compete with tyrosine for hydroxylation by tyrosinase and interfere with the completion of melanin synthesis.[41] The mechanism of cell death/apoptosis induced by these compounds is uncertain. The depigmentation process that follows exposure to phenolic agent 4-tertiary butyl phenol (4-TBP) is better understood. Occupational vitiligo in some individuals working in the rubber and tannery industries has been attributed to 4-TBP.[42] *In vitro* studies have shown that 4-TBP is specifically cytotoxic to melanocytes. However, 4-TBP is a tyrosine analog that binds to the catalytic site of the tyrosinase enzyme and acts as a competitive inhibitor of tyrosinase.[43] The cytotoxic effects were found to be independent of tyrosinase activity.[43,44] 4-TBP is shown to activate apoptosis in melanocytes manifested by plasma membrane blebbing, DNA fragmentation, and phosphatidylserine relocalization.[44]

Okadaic acid treatment of melanocytes revealed that annexin-positive melanocytes were more in unstable vitiligo as compared to stable vitiligo and controls, whereas annexin-positive cells were almost negligible in stable vitiligo and controls.[16] As an early signal, cells undergoing apoptosis display surface expression of phosphatidyl serine and are opsonized for phagocytosis by phosphatidyl serine receptor expressing macrophages.[45] Caspase 3 expression also increases more in unstable vitiligo melanocytes as compared to stable vitiligo and controls. Caspases are crucial mediators of programmed cell death (apoptosis). Among them, caspase-3 is a frequently activated death protease, catalyzing the specific cleavage of many key cellular proteins.

SUMMARY

In this review, we summarized all the theories for NSV with focus on a new integrated theory which takes into account melanocyte detachment and transepidermal elimination, neural-biochemical, and autoimmune hypotheses. As there can be different pathomechanisms for different subtypes of vitiligo, melanocytorrhagy can play an important role in some subsets of vitiligo. This new theory proposes that NSV is a primary melanocytorrhagic disorder with altered melanocyte responses to friction and possibly other types of stress, inducing their indolent detachment and subsequent transepidermal loss. Adhesion system of melanocytes is far weaker than the system which firmly holds epidermal keratinocytes to the basement membrane. Dendrites are critically important for melanosome transfer, because one melanocyte contacts several keratinocytes in the epidermis through dendritic cell processes. In NSV melanocytes, loss of dendricity induced either by oxyradicals (impaired redox status hypothesis) or by increased release of catecholamines (neural biochemical hypothesis) increase melanocytes transepidermal loss. This loss of dendricity could also affect melanosome transfer and contribute to depigmentation. Besides defective adhesion and dendritic loss, other abnormalities may lead to a decrease in the frictional resistance of melanocytes in NSV and eventually to their detachment. After their detachment, melanocytes undergo transepidermal elimination. During the transepidermal migration, melanocyte early apoptosis cannot be detected in sections when they are in a suprabasal position. Damaged melanocytes may release melanosomal antigens during their transepidermal migration and could induce, if self tolerance is broken for any reason, an immune response.

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