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Involvement of immunological factors in the pathogenesis of vitiligo

Vitiligo is an acquired depigmenting skin disease, characterized by the presence of white, well demarcated, often symmetrically distributed patches on the skin and depigmented overlying hair. Even though the disease is asymptomatic, it often brings about psychological problems and decreases the patient's quality of life because of its disfiguring effect. Vitiligo affects approx. 0.5 to 1% of the world's population, both males and females, at any age and it is more commonly observed in the black individuals. It is believed that vitiliginous skin lesions are due to the absence or destruction of melanocytes (1, 13).

Melanocytes are found in the majority of tissues but are most abundant in the epidermis and dermis as well as in the hair follicles, the eye, around the blood vessels, in the peripheral nerves, sympathetic paths and in the lining of the coelomic cavity. The process of melanin production (melanogenesis) takes place in the melanocytes' cytoplasmic organelles called melanosomes. Each melanocyte supplies melanin to approximately 36 surrounding keratinocytes. Melanin is derived from an amino acid – tyrosine. Tyrosinase, a copper-containing enzyme, and tyrosinase-related proteins: TRP-1, TRP-2, are involved in the melanin synthesis (1).

Even though many studies on the etiology of vitiligo have been conducted so far, its pathogenesis still remains unknown. The researchers have been trying to detect those immunological and non-immunological factors which are responsible for this skin condition.

GENETIC FACTOR

Vitiligo is likely to appear in about 30 to 40% of the patient's family members, which confirms the belief that vitiligo is a hereditary genetic defect. The condition has been observed in monozygotic twins (1). An autosomal dominant pattern of inheritance is likely to occur in the majority of cases. The transmission seems to be complex and polygenic with variable expression of the involved genes (VITI1, catalase, tenascin, FOXD3) (9). Several HLA changes have been reported in vitiligo patients such as DR4, DR6, B13, DQw3, DRW6, DRW52, A2 and A30 (1, 2, 9, 13). The present theory suggests that genetic factors make melanocytes more damage prone, which is the predisposing factor in the development of vitiligo (3).

POTENTIAL CAUSES OF VITILIGO

Among possible pathomechanisms the most common are: 1) autoimmune hypothesis, 2) auto-cytotoxic hypothesis, 3) neuronal hypothesis (2, 4).

Autoimmune hypothesis. Autoimmune hypothesis assumes that vitiligo can be an autoimmune disease. It is due to the fact that vitiligo often coexists with other autoimmune diseases such as *alopecia areata*, autoimmune thyroid disease, Addison's disease, pernicious anaemia, *diabetes mellitus*, *myasthenia gravis* (2). Moreover, in vitiligo patients the following abnormal antibodies have been distinguished: anti-melanocyte antibody (AMA), rheumatoid factor (RF), complement 3 and 4 (C3 and C4), antinuclear antibody (ANA) and organ-specific autoantibodies to the thyroid, gastric parietal cells and adrenal tissue (1, 2). The antibodies specific to melanocyte cell-surface antigens are found in most vitiligo patients and their presence and serum level correlate with the area of depigmented skin and the activity of the disease. They are present in 50% of the population with minimal skin changes (less than 2% of the body surface) and in 93% of those with more than 5% of the body surface involved. (2). Some studies have shown that in the sera of the patients with both local and generalized vitiligo antibodies to tyrosinase and tyrosinase related proteins 1 and 2 (TRP-1, TRP-2) are present and their activity is higher in active than in stable disease. Other authors have observed however, that in vitiligo antityrosinase antibodies have not been found at all (4, 9). On the other hand, the presence of antikeratinocyte antibodies is indicative of the primary involvement of this type of cells (2).

Effective immunosuppressive treatment with psoralen and ultraviolet A (PUVA) as well as with topical steroids and topical cytotoxic drugs firmly supports the autoimmune hypothesis (9).

Auto-cytotoxic hypothesis. Auto-cytotoxic hypothesis suggests that melanocytes of vitiligo patients are damage prone and increased melanocytes' activity results in their self-destruction (3). According to this hypothesis toxic compounds produced by melanocytes, including toxic melanin precursors (e.g. dopa, dopachrome, 5, 6-dihydroxyindole) and free radicals, play a role in the pathogenesis of vitiligo. Cytotoxicity in vitiligo may be due to decreased thioredoxin reductase, which is a catalasing agent present on the melanocyte's surface and functions as a scavenger of toxic free radicals. This enzyme is inhibited by calcium and its extracellular level is elevated in vitiligo patients. The process results in increased concentration of free radicals, followed by decreased activity of tyrosinase as well as decreased melanin synthesis (9, 13). It has been clinically confirmed that in contact/occupational vitiligo increased monobenzylether of hydroquinone may cause leukoderma (9). Disturbed epidermal and systemic metabolic pathways of biopterins and catechols have been reported. Impaired metabolism of the tetrahydrobiopterin (cofactor for the hydroxylation of phenylalanine to tyrosine), which leads to epidermal accumulation of 6BH4 and 7BH4, has been observed all over the epidermis in vitiligo patients. These metabolites act as melanin synthesis inhibitors and oxidative stress boosters (3). Moreover, they increase production of catecholamines which also support oxidative stress and compete with tyrosine on the biochemical pathway. It has been shown that vitiliginous keratinocytes produce 4 times more norepinephrine and 6.5 times less epinephrine than within the area of the healthy skin. It is reflected in increased norepinephrine serum level as well as increased urinary concentration of homovanillic and vanil mandelic acids (13).

Neuronal hypothesis. Taking into account the same melanocytes and neurons' embrional origin, the neuronal hypothesis claims that secretion of some toxic for melanocytes, neurochemical mediators at the peripheral nerve endings in the skin brings about melanogenesis inhibition (1, 13). Increased levels of catecholamine released from nerve endings in the skin or its catabolites may lead to the destruction of melanocytes. In vitiliginous skin some features of axon degeneration have been found (9).

Segmental distribution of skin lesions, observed in certain forms of vitiligo, is supportive of this theory (9). Moreover, it has been proven that a severe emotional event triggers the onset of the disease. There is also some evidence that vitiligo is more likely to develop in patients with neurological disorders such as: peripheral nerve injury, viral encephalitis, multiple sclerosis (9).

MELANOCYTE AND KERATINOCYTE RELATIONSHIP

Histopathological studies of affected skin areas reveal a complete absence of melanocytes, whereas according to various authors in the perilesional skin the number of melanocytes is normal or reduced. In the margins of the active lesions enlarged and fragmented melanocytes are usually found (2). Moreover, the electrone microscopic analysis has shown vacuolar degeneration in basal and suprabasal layers, swelling of membrane-bound organelles and cytoplasm condensation in keratinocytes, especially in perilesional skin (3, 6). The data suggest that malfunctioning of keratinocytes is responsible for the absence of melanocytes as well as for impaired melanocytes' growth (6). This concept is strongly supported by the notion that melanocytes and keratinocytes are closely related and together they form a functional complex called epidermo-melanic unit. Keratinocytes produce various cytokines which play an important role in melanocyte migration, proliferation, differentiation and melanin synthesis. Impaired keratinocytes do not produce a sufficient amount of specific melanocyte growth factors such as: granulocyte-macrophage colony-stimulating factor (GM-CSF), stem cell factor (SCF), basic fibroblast growth factor (bFGF) and their deficiency leads to melanocytes' apoptosis (7, 13). Decreased expression of Bcl-2, p53 and increased expression of proapoptotic Bax, FLIP, 3, 8, 9 caspases have been observed (3).Other cytokines synthesized by keratinocytes, including interleukin 1α (Il-1α), interleukin 6 (Il-6), tumor necrosis factor α (TNFα) and transforming growth factor β (TGF-β), are paracrine inhibitors of human melanocyte proliferation and melanogenesis (7, 13). *In vivo* studies have revealed that TNF-α inhibits melanogenesis through an inhibitory effect on tyrosinase and tyrosinase-related protein 1 (6). Moreover these pro-inflammatory cytokines promote the expression of intercellular adhesion molecule 1 (ICAM-1) initiating lymphocyte recruitment (3, 6). In perilesional, non-lesional and healthy skin the level of keratinocytes' cytokines is normal (6).

CELLULAR IMMUNITY

Plausible theory on vitiligo etiology states that immune tolerance to self-melanocyte antigens is broken in this condition (5). Recent studies have revealed that antibodies in vitiligo generally react with intracellular antigens such as VIT40, VIT75, VIT90. It is highly probable that the melanin-concentrating receptor-1 (MCR1) is a target for vitiligo autoantibodies (2, 11). It is still unknown how the immune system is primarily attacked and compromised on the cellular level. *In vitro* studies have shown that antibodies can kill pigment cells by complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity (ADCC) (2).

It has been suggested that keratinocytes can present melanocyte antigens in a major histocompatibility complex II (MHC class II) restricted manner whereas according to other researchers keratynocytes initiate the process and are responsible for it (10). What is more, it is worth noting that melanocytes are also capable of presenting antigens to T-cells in MHC class II restricted manner and starting T-cells infiltration (10). It has been shown that melanocytes in perilesional skin express higher levels of MHC class II and ICAM 1 in comparison with the normal skin (4).

Symmetrical distribution of the lesions is suggestive of some role of clones of lymphocytes with affinities for specific areas of the skin (8). Expression of the skin homing receptor CLA (cutaneous lymphocyte antigen) is a characteristic feature of the T cells (11).

Histopathological studies have revealed a mild mononuclear cell (lymphocytes and macrophages) infiltrate in the margins of lesional and normal pigmented skin (9, 11). The infiltrate is seen only in an active stage of the disease and is scarce. In early skin changes the lymphocytic infiltration coexists with melanocytes suggesting a role of inflammatory infiltrate in melanocyte degeneration (10). According to immunohistopathological studies an infiltrate is composed of CD3+ (T-cells), CD4+ (helper T-cells) and CD8+ (cytolytic T-cells) lymphocytes, CD68+ macrophages and Langerhans' cells (LC) (2). The most common infiltrate cells are lymphocyte CD8+ specific for melanocytic antigens that have probably escaped clonal deletion in the thymus. The CD8+/CD4+ ratio is increased. CD8+ lymhocytes can play a role in melanocytes' apoptosis by the activation of granzyme/perforin pathway (11). T cells express activation molecules such as interleukin 2 receptor (Il-2R- CD25), MHC II and HLA-DR. Elevated levels of soluble interleukin-2 receptors have been found in the sera and in the tissue fluids as well (10). Perilesional T-cell clones presented prevalent type I T-cells releasing IFN-γ, TNF-α (14). The IFN-γ increases infiltration of T-cells to the skin by increasing ICAM-1 expression (10). These cytokines can also initiate apoptosis. It is suggested that a therapy aiming at increasing the level of type II T-cells, which secrete Il-4, Il-5, Il-10, Il-13, could be highly effective in vitiligo treatment (5). The autoimmune response in vitiligo is also attributed to the absence of T regulatory cells (T regs) that act as suppressors of the immune response by secreting Il-10 and TGF-β. The lack of T regs allows for cytotoxic T cells' proliferation as well as their migration, thus resulting in progressive depigmentation (11).

There is some evidence that LC can be involved in vitiligo pathogenesis. Decreased number of epidermal LC has been observed in active and repigmenting vitiligo while LC number remains normal in stable disease. It can be due to destruction of the LC by cytotoxic factors or their migration to the regional lymph nodes (10). Other authors have presented contradictory results which suggest that LC may be regular in number or they may even be more densely distributed in vitiliginous skin (4).

As far as monocytes are concerned, the increased production of Il-6 and Il-8 was found. Those cytokines play a role in cell migration and B-cell activation (10).

Data concerning abnormalities in peripheral blood mononuclear cells are inconclusive because of their inconsistency (9). A decreased or normal total number and percentage of the peripheral blood lymphocytes as well as an increased number and percentage of CD4+ lymphocytes, elevated CD4+/CD8+ ratio and a lower percentage of NK-T cells and native T cells have been observed by some authors while others have obtained normal or decreased sera levels of CD4+, CD8+ as well as normal levels of natural killer (NK) cells (2, 14). Some researchers have found the presence of high frequencies of CD8+ T cells specific for melanocytic antigens which are also observed in depigmented lesions (12).

VITILIGO AND OTHER SKIN CONDITIONS

The recent clinical studies demonstrating malignant melanoma (MM) and vitiligo coexistence are suggestive of plausible similar mechanisms in the destruction of both benign and malignant melanocytes (13). *In vitro* studies have revealed that human melanoma cells secrete tyrosinase. It was confirmed in melanoma patients whose tyrosinase sera concentration was increased. Production of anti-tyrosinase antibodies can be considered as a reaction against tyrosinase in this pathological condition. It has been found that T cells isolated from vitiligo lesions react with melanoma-associated

antigens. Some authors are of the opinion that the appearance of depigmentation during the course of MM might be considered a good prognostic factor (11).

Other researchers point to the fact that vitiligo and hypopigmented mycosis fungoides may have a similar mechanism. The epidermal lymphocytic infiltrate in both conditions were composed of CD8+ cytotoxic T cells. The authors are of the opinion that cytotoxic effects of CD8+ lymphocytes may result in destabilization of SCF and lead to dysfunction and/or loss of melanocytes in the epidermis of vitiligo patients as well as those who suffer from hypopigmented *mycosis fungoides* (15).

CONCLUSIONS

The cause and pathogenic events involved in melanocytes' destruction remain unclear. Even though quite a few hypotheses have tried to explain the origin of this disease none have been successful so far. Circulating antibodies are suggestive of the humoral immune response. On the other hand, a lot of researchers stress the role of a melanocyte-specific cytotoxic T-cell immune reaction. However, further studies are needed in order to clarify vitiligo pathogenesis. Understanding the mechanisms responsible for vitiligo development will make it possible to elaborate suitable methods of treatments.

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SUMMARY

Vitiligo is an acquired disease manifested by depigmented patches of different shape and size present on the skin surface. It affects approx. 0.5–1% of the world population. Skin lesions result from the absence or destruction of melanocytes. Even though the aetiopathology of vitiligo is unknown, autoimmunological, auto-cytotoxic and neuronal hypotheses are taken into account. Positive family history of vitiligo patients confirms a role of some genetic factors. The extent and range of skin changes depend on the serum level of antibodies specific to melanocyte cell-surface antigens, which is suggestive of the humoral immune response. In the same way in the active phase vitiligo patients present cellular response mechanisms in the form of infiltrates consisting of CD4+, CD8+ T-cells as well as macrophages. Furthermore there is a close relationship between melanocytes and keratinocytes producing GM-CSF, SCF, bFGF cytokines that are indispensable for normal functioning of pigment cells. An observation that the similar pathogenic mechanism might explain depigmentation not only in vitiligo but in hypopigmented *mycosis fungoides* and *melanoma malignum* as well is worth noting. Understanding the mechanisms responsible for vitiligo development will make it possible to elaborate suitable methods of treatment.

Udział czynników immunologicznych w patogenezie bielactwa nabytego

Bielactwo jest chorobą nabytą charakteryzującą się obecnością odbarwionych, różnej wielkości i kształtu plam na skórze. Występuje u około 0,5–1% populacji na świecie. Wykwity są następstwem braku lub uszkodzenia melanocytów. Etiologia oraz mechanizm powstawania bielactwa są nieznane, chociaż rozważa się teorię autoimmunologiczną, cytotoksyczną i neurogenną. Stwierdzanie choroby u członków rodziny pacjenta przemawia za udziałem czynników genetycznych. Zależność rozległości zmian skórnych od poziomu w surowicy przeciwciał skierowanych przeciwko antygenom powierzchniowym melanocytów sugeruje znaczenie odpowiedzi typu humoralnego w patogenezie choroby. Jednocześnie u pacjentów z aktywną fazą choroby obserwuje się mechanizmy odpowiedzi komórkowej w związku z obecnością w naskórku nacieku złożonego z limfocytów T- CD4+, CD8+ oraz makrofagów. Ponadto melanocyty znajdują się w ścisłym związku z keratynocytami produkującymi cytokiny, w tym GM-CSF, SCF, bFGF, które są niezbędne do prawidłowego funkcjonowania komórek barwnikowych. Na uwagę zasługuje spostrzeżenie, że w bielactwie może występować podobny mechanizm patogenetyczny jak w hipopigmentacyjnym ziarniniaku grzybiastym i czerniaku złośliwym. Zrozumienie zjawisk odpowiedzialnych za rozwój bielactwa pomoże w opracowaniu skutecznych metod leczenia.