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LEADING ARTICLE

The Role of Tumor Necrosis Factor-α in the Pathogenesis of Vitiligo

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Abstract Vitiligo is an acquired immune disorder of the skin characterized by the presence of white depigmented macules. Its immunopathogenesis is not completely understood, but inflammatory alterations in the skin microenvironment, and particularly increased expression of the cytokine tumor necrosis factor-α (TNFα), are thought to be essential regulators of melanocyte dysfunction and death. In this article we review the evidence that implicates TNFα in the pathogenesis of vitiligo, including studies on serum and tissue levels of TNFα, TNFα gene polymorphisms, in vitro studies, and therapeutic trials using TNFα inhibitors. TNFα emerges as a complex mediator with apparently conflicting roles in vitiligo.

1 Introduction

Vitiligo is an acquired cutaneous disorder of pigmentation, with a 0.5–2 % incidence worldwide that affects males and females alike [1]. It is characterized by progressive depigmentation and the appearance of white cutaneous macules, usually with no other clinical symptoms. Clinically, it can present as a non-segmental variant, with bilateral and generalized distribution (the most common form) or as a segmental variant, involving a single body region. Depigmentation is due to the loss of functioning melanocytes [1, 2]. Multiple studies have provided evidence of various candidate genes and loci associated with vitiligo, and no single gene has been singled out as determinant for the development of the disease [2].

The immunopathological mechanisms leading to melanocyte loss are not completely understood. Autoimmune, autocytotoxic/metabolic and neural dysfunctional mechanisms have been proposed [1, 2]. Common to all of these hypotheses is an inflammatory alteration of the epidermal and dermal microenvironments. Mononuclear cell inflammatory infiltrates, changes in cytokine and chemokine balance, increased oxidative stress and changes in cellular immunity across all the involved cell subtypes appear to be primary immunopathologic events in the development of vitiligo (see Guerra et al. [1, 3] and Sandoval-Cruz et al. [1, 3] for reviews).

Altered numbers and function of T cells are proposed as participants in the development of vitiligo [4]. Increased circulating numbers of CD8+ T lymphocytes and decreased levels of regulatory T cells have been correlated to disease severity in vitiligo patients [5], although other studies have not replicated these results [6]. Nevertheless, lymphocytic infiltrates in vitiliginous lesions are a common finding, and tend to show a predominantly T-helper-1 cell (Th1) response with the release of various cytokines, including tumor necrosis factor-α (TNFα) [2, 4]. In the Smyth line animal model of vitiligo, lymphocyte imbalance and a Th1-polarized response have been confirmed as central immune alterations associated with disease activity

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[7]. However, inflammatory cells are not the only sources of cytokine production. Keratinocytes are hypothesized to produce an excess of a variety of cytokines, including TNFα, in the vitiliginous microenvironment [8], and dermal cells such as fibroblasts could be another source of TNFα production under inflammatory conditions [9].

The prominent role of TNFα in regulating the inflammatory response, from leucocyte-cell interactions to cytotoxic activity, has led to it being proposed as a central effector in the immunopathological mechanisms involved in vitiligo. However, therapeutic trials using anti-TNFα agents in treating vitiligo have shown mostly negative results, calling into question the physiopathological relevance of TNFα. In this article, we will review the evidence linking TNFα to vitiligo, and discuss a possible explanation for the conflicting results so far obtained.

2 Peripheral Blood Cells and Plasma

The determination of cytokine levels in sera or blood cells taken from patients with a disease, and comparing it with samples taken from matched controls, can lead to associations between the cytokine and disease. Unfortunately, in the case of TNFα, its general role in the acute phase response of the inflammatory cascade makes it non-specific. Thus, TNFα elevations occur in a myriad of clinical conditions. In one of the earliest studies carried out on peripheral mononuclear cells derived from patients with vitiligo, the spontaneous production of the cytokines interleukin (IL)-6 and IL-8 was found to be increased, with no changes in TNFα production [10]. In fact, TNFα production was diminished when these cells were stimulated with anti-mononuclear cell immunoglobulin. This was thought to explain the reduced inflammatory reaction found in vitiliginous lesions.

Another study failed to show increased serum levels of TNFα in 50 patients with active vitiligo, although an IL-6 elevation was indeed confirmed [11]. Similar findings were reported in another study, where IL-6 and IL-2 were increased in sera from 80 patients with vitiligo compared with normal controls, but no difference was observed in TNFα concentrations [12]. Finally, in a study of 40 patients with non-segmental vitiligo, serum levels of soluble TNF receptor I were also similar to healthy controls [6]. However, at least two studies found positive results: increased production of TNFα by peripheral mononuclear cells derived from 32 patients with active vitiligo that were stimulated with lipopolysaccharide [13], and, in the largest study to date, elevated levels of serum TNFα in 214 vitiligo patients compared with 236 unaffected controls [14]. This last study also found increased levels of TNFα messenger RNA (mRNA) expression in a subset of the vitiligo patients. Severe disease activity, vitiligo subtype and different genotypes could be important in explaining these conflicting results. Taken together, the conflicting evidence derived from studies on peripheral blood cells or serum levels of TNFα do not suggest a precise role for this cytokine in the pathogenesis of vitiligo. However, they imply a systemic proinflammatory state that may not be representative of the spectrum of vitiligo presentations, and tissue studies would seem more pathophysiologically relevant.

3 Tissue Studies

Tissue studies showing increased TNFα activity in active vitiliginous lesions compared with unaffected skin would more strongly support the idea of an important role for TNFα in depigmentation, and this is precisely what some studies have found. Tissue samples from the affected epidermis of patients with active vitiligo have been found to have increased expression of IL-6 and TNFα, by immunohistochemical methods [8]. This suggested a relevant change in the production of cytokines in the epidermal microenvironment of vitiliginous lesions. In keeping with these results, increased mRNA expression for TNFα was found to be elevated in both involved and uninvolved skin in patients with vitiligo [15], and of both TNFα and IL-6 in the epidermis of affected skin [16]. These observations have been confirmed in other studies [17], and extended to cover other hypopigmented disorders such as tinea versicolor and mycosis fungoides [18]. Furthermore, in vitro studies of cloned CD4+ and CD8+ T cells obtained from vitiliginous lesions show a striking upregulation of TNFα and a predominantly type-1-like cytokine secretion profile [19].

However, other studies point toward significant variability in TNFα immunohistochemical staining in vitiliginous lesions. In a study of eight cases, five had strong TNFα staining, whereas three did not, although TNFα staining intensity did seem to correlate to disease activity [20]. In contrast to peripheral blood and sera studies, there is substantial evidence that TNFα is increased locally in vitiliginous lesions. Considering that vitiligo is not associated with a systemic inflammatory state, but with a localized (skin) immune reaction, these conflicting findings could be easily explained.

4 Genetic Studies

Vitiligo is an acquired disease that is genetically heterogeneous. However, a role for TNFα in its pathogenesis would be supported by findings on polymorphisms associated with TNFα genes. The TNFα-308 promoter polymorphism, which leads to higher rates of TNFα transcription, has been associated with autoimmune and

inflammatory diseases. In a study of 61 patients with vitiligo, polymerase chain reaction amplification analysis of the promoter TNFα gene failed to show any difference in the G/A polymorphism at position −308 compared with age, sex, and ethnic matching controls [21]. This early negative study cast further doubts on an association between TNFα and vitiligo.

In a later study of 176 vitiligo patients, Namian et al. [22] found that the TNFα-308 G/A polymorphism was significantly more common in female vitiligo patients compared with controls, but no change in males or between vitiligo subtypes. This confirmed the heterogeneous genetic determinants of vitiligo, and suggested that genetic studies would have to control for variables other than sex and vitiligo subtype. In a recent study involving 198 vitiligo patients, the association between the TNFα-308 promoter G/A polymorphism and vitiligo was confirmed, but found only in patients with active vitiligo vulgaris: sex and age of onset were not important factors [23]. These results have been reconciled in the largest study to date. After analyzing 977 vitiligo patients, Laddha et al. [14] found that various polymorphisms (−238, −857, −863, −1031 as well as the −308 G/A polymorphism) in the promoter region of the TNFα gene were found to be significantly associated with vitiligo. That same study also showed higher TNFα transcript and protein levels in women, patients with active vitiligo, generalized vitiligo, and associations between some polymorphisms and early-age onset of disease, suggesting genotype-phenotype correlations. Laddha et al. [14] also described the association of different haplotypes with some phenotypic expressions: haplotypes AACCT, AGTCT, GATCT, and AGCCT with increased TNFα serum levels; AACCT, GATCT, GATCC, and AATCC with increased TNFα expression levels; and AACAT, AACCT, AATCC, and AATCT with early disease onset. Together, these studies support a role for TNFα promoter polymorphisms as genetic risk factors, and a role for TNFα itself in the autoimmune pathogenesis of vitiligo.

5 Mechanisms

TNFα is thought to participate in the immunopathogenesis of vitiligo by inducing melanocyte dysfunction and death through various mechanisms. TNFα has been demonstrated to be proapoptotic in various tissues and cell types. TNFα is also known to be induced by and act as an inducer for nuclear factor kappa-B (NF-κB), a transcription factor involved in inflammatory and pro-survival gene promotion. Death receptors belonging to the TNF receptor superfamily, such as TNF-related apoptosis-inducing ligand (TRAIL), participate in the induction of programmed cell death and play important roles in the immunopathogenesis of skin diseases [24]. TRAIL promotes apoptosis of primary human melanocytes in vitro by activation of caspases and cleavage of vital proteins [25], and melanocytes exposed to chemical stressors show increased TRAIL expression and promote dendritic cell-mediated melanocyte death [26].

Melanocyte function, including proliferation, differentiation and immunologic susceptibility to cytotoxicity can be altered by proinflammatory cytokines, including TNFα [27]. Adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) are overexpressed in melanocytes from vitiligo lesions, and cytokines such as TNFα can induce their expression on the surface of epidermal melanocytes [28, 29]. Moreover, TNFα is a strong inducer of ICAM-1 in both normal and vitiliginous cultured melanocytes [30]. This pathway could influence melanocyte target recognition by T cells and mediate immunologic cytotoxic damage. TNFα can inhibit melanogenesis by decreasing the intracellular levels of tyrosinase and tyrosinase-related protein 1, an abundant melanosomal glycoprotein involved in both melanogenesis and prevention of melanocyte death [31, 32]. There is also evidence that TNFα-mediated inhibition of tyrosinase activity and melanogenesis is dependent on the activation of NF-κB [33]. TNFα-treated melanocytes show marked cellular shrinking and reduced melanin production in vitro, as well as downregulation of MITF, a transcription factor essential in the regulation of melanocyte development, proliferation, death, and melanogenesis [34]. TNFα leads to a dose-dependent inhibition of melanocyte proliferation, partly through increased expression of the CXC-chemokine receptor II [35]. TNFα also leads to reduced expression of the pigment-associated antigens HMB-45 and K.1.2 in normal cultured melanocytes [36] as well as to altered immunological phenotypes.

Other mechanisms of TNFα-induced alterations in melanogenesis have been uncovered. Melanocyte-stimulating hormone receptor (MSH-R) and melanocortin-1 receptor (MC1-R) are known inducers of melanogenesis, capable of inducing the expression of melanin synthase, modulating pigmentation and melanocyte survival in normal and pathological conditions (see Slominski et al. [37] for an excellent review). In vitro studies of normal human melanocytes have shown that TNFα downregulates MSH-R binding activity and reduces the expression of MC1-R mRNA [38]. TNFα also reduces the expression of gp87 in melanoma cells, a melanosomal protein involved in melanogenesis [39]. A recent study shed further light on the effects of TNFα in inflammation-associated pigmentation changes. Using normal human melanocytes, Wang et al. [40] showed that TNFα could stimulate the melanoma mitogens IL-8 and CXCL1, inhibit pigmentation-related signaling and melanin production, and increase the production of β-defensin 3, an antagonist for MC1-R.

A percentage of cultured normal melanocytes respond to TNFα with apoptosis, but melanocytes with high basic NF-κB binding activity do not show TNFα-induced NF-κB activation and are apoptosis resistant [41]. Apoptosis-sensitive melanocytes in turn show reduced melanogenesis. This suggests that melanocytes with impaired melanogenesis have altered NF-κB signaling that leads to susceptibility to TNFα-induced apoptosis. Impaired phosphatidylinositol 3-kinase/serine/threonine protein kinase activation followed by reduced NF-κB activation under increased TNFα levels was demonstrated as a mechanism for keratinocyte apoptosis in vitiligo as well [42]. Indeed, human vitiliginous keratinocytes treated with TNFα show increased apoptosis due to an impaired phosphatidylinositol 3-kinase/protein kinase B signaling pathway [43].

The participation of altered redox homeostasis in the pathogenesis of vitiligo has been reviewed by Laddha et al. [44] and Glassman [4]. The generation of a redox imbalance and overproduction of reactive oxygen species (ROS) such as nitric oxide (NO) and hydrogen peroxide (H_2O_2) could represent another possible mechanism of TNFα-induced melanocytotoxicity. The intracellular levels of H_2O_2 and other ROS increase in various cell systems in response to TNFα stimulation [45]. In studies of in vitro human primary keratinocytes, TNFα dose-dependently and rapidly induces ROS generation, and ROS further mediate TNFα-mediated production of inflammatory cytokines [46]. In human skin-derived cultured fibroblasts, incubation with TNFα led to increased production of H_2O_2 and other ROS [47]. H_2O_2 can also act as a messenger in the TNFα-dependent activation of NF-κB [48].

TNFα is known to induce NO production in cultured melanocytes through upregulation of inducible NO synthase [49]. A similar mechanism has been observed in cultured keratinocytes, which show TNFα-induced NO production leading to increased apoptosis [50]. A TNFα-mediated altered redox state in the skin could then lead to membrane lipid alterations and increased mitochondrial production of ROS, resulting in melanocyte apoptosis in vitiligo [1, 51]. The complex interplay between oxidative stress and immune mediators such as proinflammatory cytokines and T cells suggest a central role for oxidative stress—autoimmunity-mediated melanocyte loss in vitiligo as well [44].

The TNFα-associated alterations in melanocytes reviewed here (Fig. 1), coupled with evidence of increased TNFα levels in affected skin and TNFα gene polymorphism studies, point towards a causal role for TNFα in the immunopathogenesis of vitiligo. Studies involving modulating TNFα as a therapeutic strategy would seem warranted, and in fact some have been reported in the literature.

6 Treatment Studies

The expression of TNFα mRNA is decreased in lesions treated with topical tacrolimus, a finding associated with clinical improvement [15]. Although this could simply reflect a general anti-inflammatory effect induced by tacrolimus, a possible role for TNFα in the pathophysiology of vitiligo has led to the evaluation of anti-TNFα therapies as possible treatments [52]. A single case report of a patient with ankylosing spondylitis treated with infliximab (a monoclonal antibody against TNFα) described remarkable improvement in concomitant vitiligo lesions

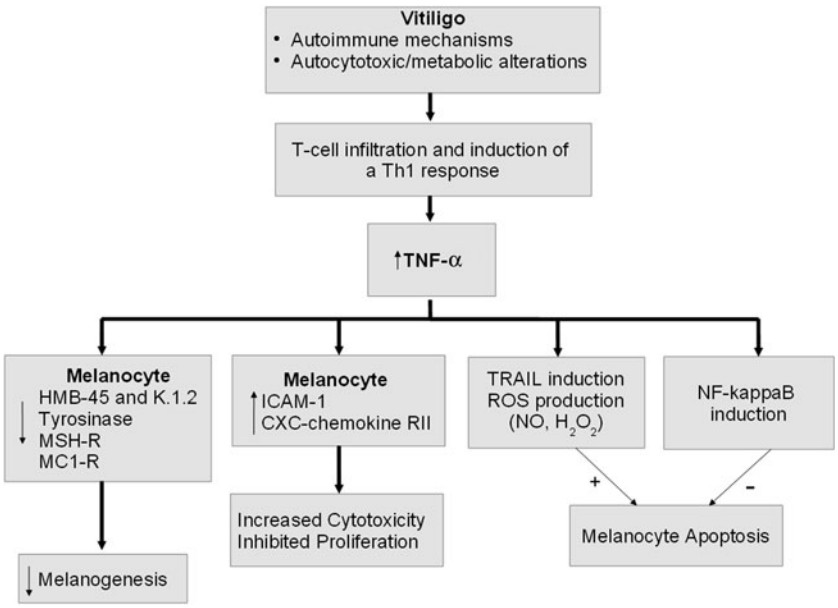

Fig. 1 Mechanisms of TNFα-mediated alterations in melanocyte function in vitiligo. *CXC-chemokine RII* CXC-chemokine receptor II, *H_2O_2* hydrogen peroxide, *ICAM-1* intercellular adhesion molecule-1, *MC1-R* melanocortin-1 receptor, *MSH-R* melanocyte-stimulating hormone receptor, *NF-kappaB* nuclear factor kappa B, *NO* nitric oxide, *ROS* reactive oxygen species, *Th1* T-helper-1 cell, *TNF-α* tumor necrosis factor-α, *TRAIL* TNF-related apoptosis-inducing ligand, ↑ indicates increased, ↓indicates decreased

Table 1 Effects of anti-tumor necrosis factor-α treatments in patients with vitiligo and patients treated for other conditions who developed vitiligo

Condition (no. of patients)	Intervention	Outcome	References
Ankylosing spondylitis and vitiligo (1)	Infliximab (350 mg, 8 doses in 1 year)	Improvement	[53]
Psoriasis and vitiligo (1)	Etanercept (100 mg/week for 12 weeks, then 25 mg/week for 12 weeks)	Mild improvement	[54]
Vitiligo (4)	Etanercept (100 mg/week for 12 weeks, then 25 mg/week for 4 weeks)	No change	[55]
Refractory vitiligo (1)	Etanercept (100 mg/week for 1 year)	Improvement	[20]
Vitiligo (6)	Etanercept (as in psoriasis)	No change	[56]
	Infliximab (as in psoriasis)		
	Adalimumab (as in psoriasis)		
Vitiligo (1)	Infliximab (5 mg/kg, 6 doses over 24 months)	Worsening; developed psoriasiform dermatitis	[58]
Rheumatoid arthritis (1)	Infliximab	Developed vitiligo	[59]
Ulcerative colitis (1)	Infliximab	Developed vitiligo	[60]
Psoriatic arthritis (1)	Infliximab	Developed vitiligo, vasculitis, thyroiditis	[61]
Psoriasis (1)	Adalimumab	Developed vitiligo	[62]

[53], and a patient with psoriasis and vitiligo treated with etanercept (TNFα receptor antibody) showed mild improvement in his concomitant vitiligo [54]. However, another study failed to show any clinical efficacy of etanercept in treating vitiligo in four patients [55], and a recent study involving six patients with vitiligo treated with infliximab, etanercept or adalimumab (humanized monoclonal antibody against TNFα), with the specific aim of treating their vitiligo, failed to show any efficacy [56]. Treatment individualization, as in using anti-TNFα agents in patients with known tissue overexpression of TNFα, could lead to better outcomes, as a recent study has shown [20].

The issue is further complicated by findings that anti-TNFα therapy can lead to the appearance of immune-mediated skin lesions, including vitiligo [57]. In fact, a case of a patient with vitiligo treated with infliximab who developed psoriasiform dermatitis and worsening vitiligo was reported recently [58]. Infliximab and adalimumab therapy have been associated with the appearance of de novo vitiligo in other clinical settings, such as rheumatoid arthritis, ulcerative colitis, and psoriatic arthritis [59–62]. Although the mechanism of anti-TNFα-induced vitiligo is unknown, some authors have speculated that paradoxical induction of autoimmunity against melanocytes might be involved [59]. Indeed, there is convincing evidence showing that anti-TNFα therapy can lead to development of auto-antibodies and new-onset of autoimmune disorders [57, 63]. Another study showed increased TNFα expression in involved skin, but *after* treatment with narrow-band ultraviolet B therapy in 20 patients with vitiligo [64]. There is little support of therapeutic efficacy for any type of anti-TNFα treatment in vitiligo, which is unexpected considering the tissue studies discussed above (summarized in Table 1).

7 A Dual Role for Tumor Necrosis Factor-α

Tissue and in vitro studies seem to suggest a role for TNFα in the immunopathogenesis of vitiligo. Serums studies, and to a lesser extent genetic studies, are conflicting. However, treatment studies do not support the use of TNFα inhibition as an effective therapeutic strategy. Insights on the dual function of TNFα could partly explain these discrepancies.

The inflammatory effects of TNFα and its role in tissue injury in diverse pathologies are well known. However, TNFα has also been shown to induce tissue repair and to promote cell survival, as in the case of central and peripheral nervous system injuries [65, 66]. In cancer biology, TNFα is well known to possess anti-cancer activity, but recent findings suggest that it may also promote cancer development and progression [67]. In wound repair studies, an initial inflammatory response mediated by cytokines such as TNFα ultimately leads the way towards adequate wound healing [68]. In the heart, TNF receptor activation can lead both to myocyte injury and to increased resistance to apoptosis, depending on the cellular microenvironment [69]. The complexity of TNFα signaling, and its dual effects in both injury and repair, could explain the clinical failure of TNFα inhibition in diseases where pre-clinical studies strongly suggested a role for

TNFα in their pathogenesis, such as heart failure, multiple sclerosis, asthma, or dermatomyositis [70].

The transcription factor NF-κB could be important for explaining these observations. TNFα is able to induce NF-κB in a complex crosstalk of TNF-receptor signaling [71], and NF-κB is known to be a key regulator of the expression of cell survival and repair genes. In the case of vitiligo, reduced NF-κB activation is known to result in increased susceptibility to apoptosis in vitiliginous keratinocytes [43], and NF-κB can mediate the transcription of antiapoptotic factors which may block TNFα-induced apoptosis in melanocytes [36]. The finding of increased TNFα in affected skin of patients with vitiligo after treatment with ultraviolet B also suggests a role for TNFα in repigmentation [64].

Although TNFα-induced inhibition of melanogenesis is well established, TNFα could also have a dual role in the pigmentation process. TNFα is constitutively expressed in both normal and perturbed dermis and epidermis, which suggests it plays a role in skin homeostasis [72]. Endothelin-1 (ET-1) and stem cell factor (SCF) are melanogenic factors produced in response to various stimuli, capable of modulating inflammation-induced hyperpigmentation [37]. Both ET-1 and SCF mRNA has been found to be reduced in vitiliginous skin in correlation with increased levels of TNFα [16]. However, studies have shown that TNFα can stimulate the production of ET-1 in skin, leading to enhanced pigmentation in seborrhoeic keratosis [73]. Furthermore, TNFα is able to stimulate SCF production in normal human keratinocytes [74]. In discussing the role of an altered redox state in vitiligo we reviewed evidence showing that TNFα can induce NO production in melanocytes. NO is also melanogenic factor produced from cells surrounding melanocytes in response to ultraviolet irradiation, and also plays a role in intracellular signal transduction pathways regulating melanogenesis [37, 75]. Thus, TNFα could play a dual role in vitiligo as well, acting on different cell populations and at different timepoints, both in promoting melanocyte injury and inhibiting melanogenesis on one hand, and promoting their survival and function on the other (Fig. 1).

8 Conclusion

The immunopathogenesis of vitiligo is complex and not completely understood. Plasma and peripheral blood cell studies of TNFα levels in vitiligo have given conflicting results. Genetic and tissue studies do suggest that TNFα might play an important role in the development of vitiliginous lesions, although with some heterogeneity as to its precise relevance. Furthermore, molecular mechanisms of TNFα-mediated melanocyte dysfunction and death have been uncovered using in vitro methods. A dual role for TNFα could explain the lack of clinical effectiveness of TNFα inhibition therapy in vitiligo. However, abandoning this promising strategy would be premature. Treatment individualization, patient selection (possibly including genotypic or phenotypic variables) as well as taking into account different time periods in treatment (acute, subacute, or chronic), could lead to positive results, and further studies will be required to clarify these issues.

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