

Tumour necrosis factor- α inhibition can stabilize disease in progressive vitiligo

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Summary

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Tumour necrosis factor (TNF)- α , a proinflammatory cytokine central to many autoimmune diseases, has been implicated in the depigmentation process in vitiligo. We review its role in vitiligo by exploring its pro- and anti-inflammatory properties and examine the effects of blocking its actions with TNF- α antagonist therapeutics in reports available in the literature. We found that TNF- α inhibition halts disease progression in patients with progressive vitiligo but that, paradoxically, treatment can be associated with *de novo* vitiligo development in some patients when used for other autoimmune conditions, particularly when using adalimumab and infliximab. These studies reinforce the importance of stating appropriate outcomes measures, as most pilot trials propose to measure repigmentation, whereas halting depigmentation is commonly overlooked as a measure of success. We conclude that TNF- α inhibition has proven useful for patients with progressive vitiligo, where TNF- α inhibition is able to quash cytotoxic T-cell-mediated melanocyte destruction. However, a lingering concern for initiating *de novo* disease will likely prevent more widespread application of TNF inhibitors to treat vitiligo.

What's already known about this topic?

- Increased levels of tumour necrosis factor (TNF)- α are present in active vitiligo lesions.
- Upon TNF- α inhibition in vitiligo, anti-TNF- α agents have been labelled as ineffective, as repigmentation was not achieved.

What does this study add?

- In patients with progressive disease, TNF- α inhibition may provide a means to halt depigmentation in vitiligo.

A dual role for tumour necrosis factor- α in vitiligo

Tumour necrosis factor (TNF)- α , also known as cachectin, is a polypeptide hormone¹ that plays a role in inflammatory, infectious and autoimmune processes in human disease.^{2,3} The TNF gene is located on chromosome 6, and its production is regulated at the transcriptional and post-transcriptional levels.⁴ TNF- α exists in two forms: a transmembrane protein and a soluble protein. Both are biologically active *in vivo*.⁵

While TNF- α is produced by a diverse array of resident tissue cells including melanocytes,^{6–8} the most prominent producers are migratory immune cells such as activated macrophages² and activated T cells.⁹ TNF- α binds to, and thereby acts through, receptors TNFR1 and TNFR2. TNFR1 receptors are expressed by a wide variety of cell types^{10,11} and mediate apoptosis through a caspase 3-dependent pathway.¹² The cytoplasmic domain of TNF receptor I has a death domain, which recruits TNF receptor-associated death domain (TRADD), Fas-associated death domain (FADD) and FADD-like

interleukin-1 β -converting enzyme (FLICE). This leads to caspase-3 activation, which then induces apoptosis by prompting degradation of multiple proteins.¹²

TNFR2 receptors are far more rare, and expression is limited to fewer cell types including lymphocytes.¹³ TNFR2 does not contain a death domain and therefore cannot mediate apoptosis.¹⁴ Instead, through TNFR2 receptors, TNF- α can activate and stimulate the proliferation of T cells.¹⁴ TNF depletion in the presence of specific TNFR2 agonists may be an ideal way to treat autoimmune disease, as TNFR2 agonism results in selective regulatory T cell (Treg) activation.¹⁵ Interestingly, melanocytes can express both TNFR1 and TNFR2 receptors in response to stress.¹⁶

TNF- α has been implicated in the pathophysiology of several autoimmune diseases, particularly within the fields of rheumatology, gastroenterology and dermatology. Indeed, TNF- α levels are elevated at prime sites of inflammation in certain autoimmune diseases, including the synovial fluid of patients with rheumatoid arthritis¹⁷ and the synovium^{18,19} and sacroiliac joints²⁰ of patients with ankylosing spondylitis. In Crohn disease, TNF- α is thought to increase intestinal epithelial permeability, thereby interrupting the gut barrier and driving inflammation.²¹ The proinflammatory properties of TNF- α stem from its activation of the proinflammatory cytokines interleukin (IL)-1 and IL-6²² and of numerous nuclear transcription factors, most importantly nuclear factor (NF)- κ B, which is involved in maintaining inflammatory responses.²³

The CD4⁺/CD8⁺ ratio is markedly decreased in perilesional skin of actively depigmenting patients.²⁴ In vitiliginous skin, perilesional T cells of the CD8⁺ and CD4⁺ subsets secrete predominantly type 1 cytokines, including TNF- α .²⁵ TNF- α plays an important role in the development of cytotoxic T lymphocytes (CTLs),²⁶ which are implicated in disease initiation in vitiligo, and it enhances activated lymphocytic expression of interferon (IFN)- γ ,²⁷ a cytokine implicated in the depigmentation process in vitiligo.²⁸ As patients with vitiligo exhibit reduced mRNA levels for full-length and soluble cytotoxic T-lymphocyte-associated protein 4 compared with controls, and these receptors negatively regulate T-cell function, patients are more likely to experience the excessive T-cell responses implicated in vitiligo pathogenesis.²⁹

Recent evidence suggests that TNF- α might possess anti-inflammatory properties as well. For example, TNF- α activates and induces proliferation of Tregs *in vivo*.^{30–32} Tregs are responsible for immune homeostasis. They suppress the activity of autoreactive T cells that escape clonal deletion in the thymus, such as those reactive against melanocytes in vitiligo, dampening inflammation as needed.³³ Their abundance is markedly reduced in skin of patients with vitiligo.³⁴ Given these functional characteristics, TNF- α potentially plays both harmful and protective roles in vitiligo, by activating the cytotoxic T cells that are detrimental to melanocytes, and stimulating Tregs, respectively.

Tumour necrosis factor- α inhibitors: current applications in clinical practice

TNF- α antagonists are therapeutic agents that have proven efficacious in the treatment of certain autoimmune diseases. The

five main TNF- α antagonists approved by the US Food and Drug Administration and currently used in clinical practice are infliximab, etanercept, adalimumab, golimumab and certolizumab pegol.

Adalimumab and golimumab are anti-TNF monoclonal antibodies with a human variable region and a human IgG1 constant region. Infliximab is a humanized anti-TNF monoclonal antibody, containing a mouse variable region and a human IgG1 constant region. All three of these agents act as decoy receptors to bind TNF- α so the cytokine cannot bind and act through its true receptors.³⁵

Etanercept is a fusion protein of human TNFR2 and a truncated human IgG constant region. It contains duplicate domains of the human TNFR2 receptor, which is located on the lymphocyte surface and promotes T-cell activation.³⁶ This allows competitive binding of TNF- α , thereby preventing binding of TNF- α to true TNFRs on cell surfaces.³⁷ Etanercept was found to bind soluble TNF- α with a greater affinity than infliximab or adalimumab, although all three agents bound transmembrane TNF- α with similar affinities.³⁸

Finally, certolizumab is a humanized, anti-TNF- α monoclonal antibody covalently attached to polyethylene glycol chains (PEGylated), which binds TNF- α with great affinity. Unlike other TNF- α inhibitors, certolizumab does not contain an Fc region.³⁹ Pegylation provides a longer half-life for the drug by preventing its clearance from the plasma, thereby allowing for less frequent dosing.⁴⁰ All agents inhibit both soluble and membrane-bound TNF, but have different structures and may have different mechanisms of action.^{41–43} These TNF- α antagonists have proven successful in treating ankylosing spondylitis,^{44,45} rheumatoid arthritis,^{46,47} psoriasis,^{48–51} psoriatic arthritis^{52,53} and Crohn disease,^{54,55} supporting the pathological role of TNF- α in these diseases.

Tumour necrosis factor- α , melanocytes and vitiligo: the rationale for tumour necrosis factor- α antagonists in the treatment of vitiligo

Vitiligo is a cutaneous disease presenting with a loss of epidermal melanocytes.⁵⁶ Vitiligo is a multifactorial disease, but the Vitiligo European Task Force reached a consensus that autoimmunity plays at least some role in every type of vitiligo.⁵⁷ T-cell-mediated autoimmunity in particular plays a prominent role in disease progression, as skin-infiltrating cytotoxic T lymphocytes are reactive with melanocytes.⁵⁸ The ultimate destruction of melanocytes may be due, in part, to an imbalance in the local cytokine environment. An abundance of proinflammatory cytokines such as TNF- α has been reported in vitiligo-affected skin as compared with nonlesional skin.^{59–61}

Multiple triggers, including infectious agents, might precipitate such cytokine imbalances. In fact, recent evidence suggests that viral infections might trigger melanocyte secretion of proinflammatory cytokines including TNF- α , which ultimately results in disease development.⁶² TNF- α was proposed as a contributor to depigmentation in vitiligo after discovery of increased TNF- α levels in lesional vitiligo skin, particularly

in patients with active vitiligo.^{59,60,63} In fact, tissue levels of TNF- α directly correlate with vitiligo disease activity.⁶⁴

Data for TNF- α serum levels are mixed, yet the largest study to date demonstrated that serum TNF- α levels are increased in patients with vitiligo compared with healthy controls, and serum TNF- α levels are higher in patients with generalized vitiligo than in those with localized disease.^{65–67} It is likely that localized TNF- α plays an active role in vitiligo pathogenesis by activating CTLs within the skin, whereas serum TNF- α may be of some use as a biomarker of vitiligo activity in addition to the more clearly established vitiligo-associated cytokine, IFN- γ .^{68,69}

In vivo, TNF- α inhibits melanocyte proliferation and decreases melanocyte tyrosinase activity in a dose-dependent manner, and is thus expected to interfere with repigmentation.⁷⁰ The disappearance of differentiated melanocytes is a feature of vitiligo skin⁵⁶ in progressive disease, and hopes for repigmentation are based on the availability of stem cells that may be stimulated to migrate, differentiate and repigment the lesions. TNF- α can inhibit melanocyte stem cell differentiation, thereby further inhibiting repigmentation.⁷¹ TNF- α has also been shown to promote melanocyte apoptosis *in vitro*.⁷² These functional data and findings provided a rationale for employing TNF- α inhibition via the use of infliximab, etanercept and/or adalimumab for the purpose of halting depigmentation and supporting repigmentation in vitiligo.

When considering inhibition of TNF receptors rather than depleting the cytokine itself, it must be taken into account that lymphotoxin- α (LTA), also known as TNF- β , binds to receptors TNFR1 and TNFR2 as well.⁷ As increased expression of (genetic variants of) LTA has been associated with vitiligo susceptibility,⁷³ receptor inhibition may offer additional advantages over inhibiting TNF- α alone. The lack of uniformly efficacious therapeutics for vitiligo has provided ample incentive for utilizing TNF- α antagonists in a clinical setting. In

order to understand further the aetiology of vitiligo and to move towards development of more effective therapeutics, it is important to reflect on the data obtained to date.

Mixed results for tumour necrosis factor- α antagonists in the clinic

Three clinical pilot studies have been reported and are summarized in Table 1. The largest pilot study was performed with six patients with widespread, progressive vitiligo, who were randomized to treatment with infliximab, etanercept or adalimumab. Dosage was based on recommended guidelines for psoriasis. These patients had not received recent treatment for their vitiligo, and vitiligo lesions were assessed via digital photography. One patient treated with infliximab experienced further disease progression and may have required higher treatment doses to halt disease, but 83% of patients displayed disease stabilization lasting up to 6 months after cessation of therapy.⁷¹

In another pilot trial, four men with previously untreated, progressive vitiligo underwent therapy with etanercept. All patients experienced disease stabilization during their 16-week course of treatment, but post-treatment follow-up was not documented.⁷⁴ In the smallest pilot study two patients with progressive, recalcitrant vitiligo were administered etanercept, while continuing all previous vitiligo therapies. Both subjects experienced repigmentation.⁶⁴ Finally, one of us (A.B.G.) conducted a pilot study looking at the safety and efficacy of etanercept in treating vitiligo. The study did not meet the primary end point, which was the achievement of $\geq 50\%$ repigmentation at 6 months of treatment. However, it is unknown whether the subjects experienced stabilization or worsening of their disease control with etanercept, and other parameters were not measured (K.C. Webb, R. Tung, L.S. Winterfield, A.B. Gottlieb, J.M. Eby, S.W. Henning, I.C. Le Poole, unpub-

Table 1 Patients treated with tumour necrosis factor (TNF)- α antagonists for vitiligo

Age (years)/sex	Disease treated	Vitiligo disease activity	Treatment regimen	TNF- α antagonist dosing	Results	Report type	References
15–38/5 M, 1 F	Vitiligo	Progressive	Untreated for ≥ 3 months, then ETA (n = 2), IFX (n = 2), ADA (n = 2)	IFX: 5 mg kg ⁻¹ IV at weeks 0, 2, 6, then q8 wk. ETA: 50 mg SC 2 \times /wk. ADA: 80 mg SC at week 0, 40 mg SC at week 1, then 40 mg SC q2 wk	One worsened, five stabilized	PS	71
24–34/4 M	Vitiligo	Progressive	Untreated for 2 months, then ETA	50 mg SC 1 \times /wk for 12 weeks, then 25 mg 1 \times /wk for 4 weeks	Stabilized	PS	74
42/F, 46/F	Vitiligo	Progressive	Continued NB-UVB and topical calcineurin inhibitors, added ETA	50 mg 2 \times /wk, then 50 mg 1 \times /wk for ≥ 1 year	Disease stabilization and repigmentation	PS	64

M, male; F, female; ETA, etanercept; IFX, infliximab; ADA, adalimumab; NB-UVB, narrowband ultraviolet B; IV, intravenously; SC, subcutaneously; PS, pilot study; q2 wk, every 2 weeks; 2 \times /wk, two times per week.

lished results). In aggregate, 11 of 12 treated subjects experienced improvement in their vitiligo disease activity, from progressive to stable disease, or even repigmentation.

Additionally, there are several case reports detailing the effects of anti-TNF- α agents on established vitiligo, as well as the emergence of *de novo* vitiligo upon initiation of a TNF- α antagonist to treat another autoimmune condition (Table 2). Four patients with long-standing vitiligo underwent treatment with a TNF- α antagonist for another indication (ankylosing spondylitis, psoriasis and seronegative inflammatory arthritis). Three patients noted improvement in their vitiligo,^{75–77} while one patient noted rapid, marked worsening of his vitiligo after starting therapy.⁷⁸ In the latter case, the response of his ankylosing spondylitis is not reported, and the patient experienced partial repigmentation after stopping adalimumab.⁷⁸

There are also 18 reported patients who developed *de novo* vitiligo after initiating therapy with a TNF- α antagonist for nonvitiligo conditions. Seven of these patients are detailed in case reports,^{79–85} with an additional eight patients in one case series⁸⁶ and three reported in observational studies.^{87–89} In two observational studies looking at adverse cutaneous events that developed during TNF- α antagonist treatment for rheumatological conditions, one of 5437 patients developed vitiligo in one study,⁸⁹ and one of 435 patients developed vitiligo in another.⁸⁷ A third prospective observational study exclusively reported patients who developed adverse cutaneous events while undergoing TNF- α antagonist therapy. In this report, one of the 41 patients developed vitiligo.⁸⁸ Finally, one case series reports eight patients who developed vitiligo when treated with an anti-TNF- α agent for a rheumatological, dermatological or gastrointestinal ailment.⁸⁶ Among patients who developed *de novo* vitiligo, 10 patients were treated with adalimumab and eight were treated with infliximab.

In sum, 14 of 16 patients with established vitiligo experienced improvement in their vitiligo control when treated with a TNF- α antagonist for either vitiligo or another autoimmune condition. Compiling the data from all studies reported here, a total of only 18 among 5928 patients treated with a TNF- α antagonist for a nonvitiligo condition developed *de novo* vitiligo while on therapy. Nevertheless, a concern for activating vitiligo in treated patients cannot be overlooked.

Effects of tumour necrosis factor- α inhibitors on vitiligo vary by subgroup

Although the number of reported subjects is low, the results for TNF- α inhibition in the treatment of progressive vitiligo in patients without other autoimmune conditions are actually very promising. Indeed, in the three pilot studies, TNF- α antagonists proved effective in halting disease progression in most patients with progressive vitiligo. These encouraging findings are likely not confounded by adjunctive vitiligo treatments, as the subjects had either been off all other vitiligo therapies during the months prior to and during the study,^{71,74} or the patients' vitiligo had already proven refractory to therapies continued during the study

(narrowband ultraviolet B and topical calcineurin inhibitors).⁶⁴

TNF- α inhibitors have been found to increase T-cell activity in the periphery while decreasing localized (tissue) T-cell activity, as evidenced by downregulation of inflammatory genes in target tissues.⁹⁰ Unfortunately, no data are currently available to support reduced cutaneous CD8⁺ T-cell infiltration in response to treatment with TNF- α inhibitors in vitiligo. However, numerous studies describe the effects of TNF- α inhibitors on cutaneous T-cell levels in psoriasis,^{91,92} another disease in which T cells (mainly of the CD4⁺ subtype) drive disease pathology.⁹³ Decreased CD4⁺ and CD8⁺ T-cell levels were found in psoriatic plaques following treatment with etanercept as compared with untreated skin,⁹² and skin-homing CD8⁺ lymphocytes in psoriatic skin expressed markedly inhibited activation and increased susceptibility to apoptosis following exposure to infliximab.⁹¹ Meanwhile, there was no difference in absolute peripheral lymphocyte numbers following anti-TNF- α therapy.⁹² If these agents have comparable effects on CD8⁺ T-cell numbers and activation in vitiliginous skin, this could explain why patients with progressive vitiligo experienced halting of their depigmentation when treated with TNF- α inhibitors.

The duration of treatment with TNF- α inhibitors required to halt depigmentation cannot be definitively concluded due to the lack of uniformly reported treatment durations in the reported studies. However, subjects showed stabilization of disease or repigmentation by the fourth month of treatment in two of the pilot studies.^{64,74} In the four patients with established vitiligo treated with an anti-TNF- α agent for a nonvitiligo condition, it is interesting that the three patients who experienced improvement in their vitiligo had progressive disease for 11–20 years prior to anti-TNF- α exposure,^{75–77} while the patient who had marked worsening of his vitiligo had stable disease for 20 years prior to exposure.⁷⁸ These findings show that TNF- α inhibition was helpful in patients with progressive vitiligo but harmful in the one with stable disease.

Important differences exist in the immunological profile of the microenvironment in stable vs. progressive vitiligo. In actively spreading lesions, CD8⁺ T cells are grouped into clusters abutting melanocytes, whereas the concentration of skin-infiltrating CD8⁺ T cells is much lower in stable vitiligo,^{24,58,94} as active melanocyte destruction is not occurring in the latter case. In our review, TNF- α inhibitors were found to halt active depigmentation, likely because these agents interfere with T-lymphocyte activity. Moreover, whereas the activity of vitiligo impacts the extent to which the disease can be controlled with TNF- α inhibition, the data do not support a correlation between disease duration prior to anti-TNF- α exposure and treatment efficacy. As for treatment regimens, etanercept at a dose of 50 mg subcutaneously once or twice weekly for at least 2 months, with or without decreasing the dose or administration frequency during the course of treatment, was the most commonly recorded successful treatment. Efficacy was demonstrated in 10 of the 14 patients with estab-

Table 2 Patients treated with tumour necrosis factor (TNF)- α antagonists for nonvitiligo conditions

Age (years)/sex	Disease treated	Vitiligo disease activity	TNF- α antagonist	TNF- α antagonist dosing and duration	Results	Report type	References
24/M	AS	Progressive	IFX	350 mg IV at weeks 0, 2, 6 then q8 wk for 10 months	AS improved. Stabilization of vitiligo and repigmentation by 6 months	CR	75
65/M	Plaque psoriasis	Progressive	ETA	50 mg 2 \times /wk for 12 weeks then 25 mg 2 \times /wk for 12 weeks	Psoriasis improved. Stabilization of vitiligo and repigmentation at 24 weeks	CR	76
76/F	SIA	Progressive	ETA	Weekly	Repigmentation after 2 months	PP/CR	77
57/M	AS	Stable	ADA	40 mg SC q2 wk	Worsening of vitiligo after 3 months. Partial repigmentation after ADA stopped and adjuvant therapies	CR	78
61/M	RA	NA	IFX	3 mg kg ⁻¹ IV every 2 months	Developed vitiligo at 6 months. Partial repigmentation with adjuvant therapies	CR	79
51/M	UC	NA	IFX	5 mg kg ⁻¹ at weeks 0, 2, 6	UC improved. Developed vitiligo at 6 weeks. Partial repigmentation with stopping IFX and adjuvant vitiligo therapies	CR	80
55/M	PsA	NA	IFX	5 mg kg ⁻¹ IV q6 wk for 2 years, then 10 mg kg ⁻¹ q6 wk for 12 weeks	Developed vitiligo within 2 weeks after IFX dose increased. Partial repigmentation after IFX stopped	LE/CR	81
54/F	CD	NA	ADA	160 mg at week 0, 80 mg at week 2, then 40 mg q2 wk	CD improved. Developed vitiligo after 8 months	CR	82
60/M	PRP	NA	IFX	5 mg kg ⁻¹ IV then 10 mg kg ⁻¹ IV q5-6 wk for 27 months	PRP improved. Developed vitiligo after 27 months. Near complete repigmentation after IFX stopped and adjuvant vitiligo therapies	CR	83
33/M	AS	NA	ADA	40 mg q2 wk	AS improved. Developed vitiligo after 18 months	CR	84
46/F	RA	NA	IFX	q8 wk for 8 months	Developed vitiligo after 2 months	CR	85
Eight patients, mean age 38.2 \pm 11.5	Psoriasis, CD, UC, RA, AS, PsA	NA	7 ADA, 1 IFX	Unknown dose and duration	Developed vitiligo after mean treatment time of 17.4 \pm 15.8 months	CS	86
One patient	RA	NA	IFX	Unknown dose and duration	Developed vitiligo	PC	87
71/M	CD	NA	IFX	Unknown dose and duration	Developed vitiligo after 7 months	PC	88
44/M	Behçet disease	NA	ADA	Unknown dose and duration	Developed vitiligo after 6 months	PC	89

M, male; F, female; AS, ankylosing spondylitis; SIA, seronegative inflammatory arthritis; RA, rheumatoid arthritis; UC, ulcerative colitis; PsA, psoriatic arthritis; CD, Crohn disease; PRP, pityriasis rubra pilaris; NA, not applicable; IFX, infliximab; ETA, etanercept; ADA, adalimumab; IV, intravenously; SC, subcutaneously; q2 wk, every 2 weeks; 2 \times /wk, two times per week; CR, case report; PP, poster presentation; LE, letter to the editor; CS, case series; PC, prospective cohort.

lished vitiligo who experienced improvement in their vitiligo control.

Paradoxical effects have been observed with the use of TNF- α inhibitor therapeutics,⁹⁵ but we found that most patients with active vitiligo improved with these agents. Furthermore, reports of vitiligo as a cutaneous adverse event of TNF- α inhibitor therapy are sparse, and the majority of patients treated do not develop vitiligo. Indeed, a mere 18 of the 5928 reported patients treated with TNF- α antagonists for nonvitiligo conditions developed *de novo* vitiligo. However, the fact that patients developed vitiligo when exposed to these agents suggests that TNF- α might have been conferring a protective effect against depigmentation prior to its blockade. A possible explanation for this is provided below and is detailed in Figure 1. Interestingly, two patients who had been taking a stable dose of an anti-TNF- α agent did not develop vitiligo until their dose was doubled. In both cases the agent was infliximab.^{81,83} Indeed, although it is low, the risk of developing vitiligo as a cutaneous adverse event during anti-TNF- α

therapy does appear greater with the use of adalimumab or infliximab than with etanercept.

Reduced regulatory T-cell activity may contribute to disease progression in the face of tumour necrosis factor- α antagonist treatment

There exists a bipartite explanation for the unfavourable cutaneous environment in vitiligo, in that there is both an overabundance of the T cells that promote localized cutaneous inflammation (CTLs) and a shortage of the T cells that curb these inflammatory responses (Tregs). Despite stimulatory effects of the cytokine on pathological CTLs, TNF- α knockout mice were still able to develop vitiligo in a T-cell-receptor transgenic model of spontaneous disease,²⁸ suggesting that TNF- α is not the sole player in promoting depigmentation. Given that cytotoxic T cells facilitate target cell death through either granzyme/perforin- or Fas/Fas ligand (FasL)-mediated

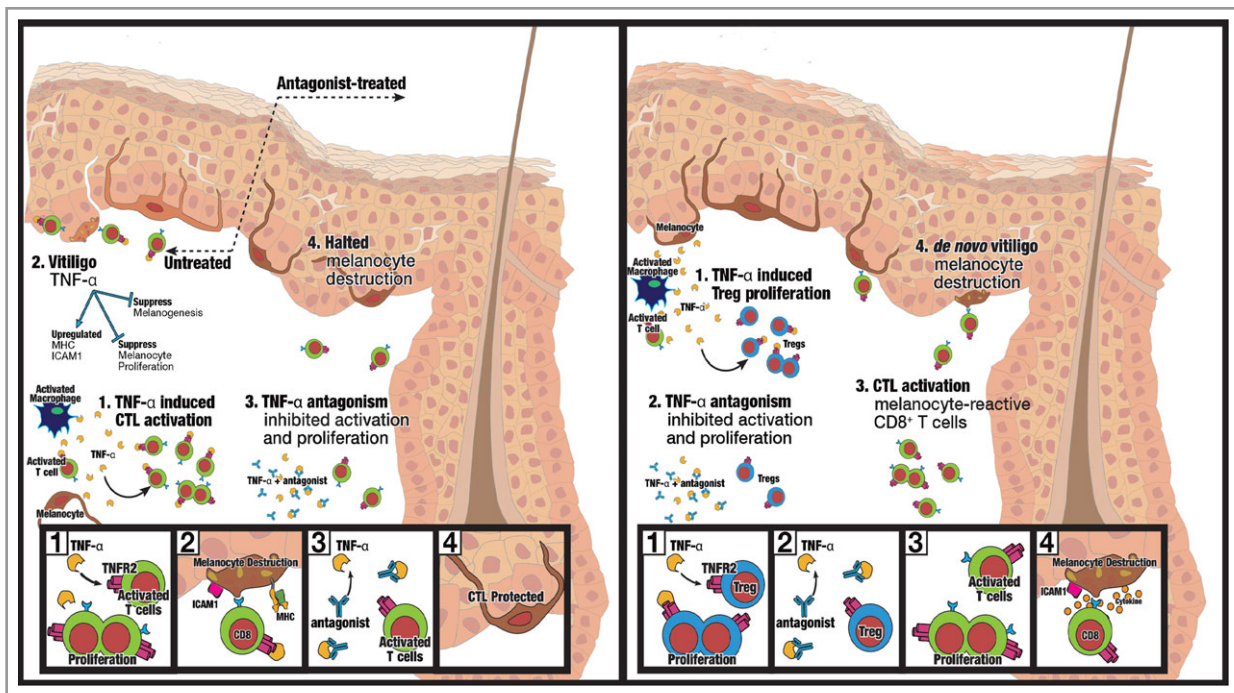


Fig 1. Effects of tumour necrosis factor (TNF)- α inhibition in patients with progressive vitiligo (left) and in patients without vitiligo (right). In patients with progressive vitiligo (left), successful halting of depigmentation is observed with the use of TNF- α inhibitors. In the skin of patients with progressive vitiligo, there is a surplus of cytotoxic T lymphocytes (CTLs) and a relative deficiency of regulatory T cells (Tregs) to suppress cytotoxic effects. With the addition of TNF- α inhibitors, there is preferential inhibition of the effects of TNF- α on CD8⁺ T-cell activation and proliferation, resulting in reduced melanocyte destruction. In patients without vitiligo, a surplus of CTLs in the skin is not observed, and Treg numbers are normal. Thus, inhibiting TNF- α instead preferentially inhibits the ability of TNF- α to promote the activation and proliferation of Tregs, resulting in removal of the skin's innate protection against depigmentation and *de novo* vitiligo development (right). On the left, in progressive vitiligo, TNF- α induces CTL activation and proliferation (Box 1), and upregulates expression of melanocyte cell-surface molecules that result ultimately in melanocyte destruction (Box 2). With TNF- α inhibitor therapy, CTL activation and proliferation is inhibited (Box 3), resulting in melanocyte survival and halted depigmentation (Box 4). On the right, in patients without vitiligo, an increase in cutaneous self-reactive CTLs is not observed. Instead, immunological homeostasis exists, with a normal amount of Tregs suppressing effects of any self-reactive CD8⁺ T cells. TNF- α induces activation and proliferation of Tregs in the skin (Box 1). When TNF- α is inhibited, Treg activation and proliferation are inhibited (Box 2), and self-reactive cutaneous CD8⁺ T cells can destroy cutaneous melanocytes (Box 3), resulting in *de novo* vitiligo development (Box 4). ICAM, intercellular adhesion molecule; TNFR, TNF receptor.

pathways,^{96,97} it would follow that one of these mechanisms facilitates melanocyte death in vitiligo. However, granzyme knockout mice were unimpaired in their ability to depigment, and melanocytes were found to be insensitive to FasL-mediated apoptosis.⁹⁸ From these studies, one can conclude that neither the TNF- α -, Fas/FasL- nor granzyme-mediated apoptotic mechanism is solely responsible for T-cell-mediated cytotoxicity. This would explain, in part, why depleting TNF- α alone can be insufficient to interfere with CTL function and depigmentation.

Instead, it is more likely that other inflammatory cytokines, such as IFN- γ , and other factors in the local microenvironment are the chief culprits in depigmentation. In fact, IFN- γ and TNF- α are observed to promote synergistically the inflammatory response, which is accredited to the cooperative interaction between the transcription factors signal transducer and activator of transcription-1 and NF- κ B.⁹⁹ Furthermore, IFN- γ , TNF- α and TNF- β induced the expression of intercellular adhesion molecule (ICAM)1 on the surface of melanocytes,¹⁰⁰ which promotes leucocyte recruitment and T-cell activation.¹⁰¹

Interestingly, melanocytes from perilesional vitiligo skin and vitiligo lesions have upregulated ICAM1 levels,¹⁰² suggesting a potentially significant role of these molecules in vitiligo disease. Indeed, genetic variants of IFN- γ and ICAM1 were shown to affect disease onset and progression of generalized vitiligo.¹⁰³ Melanocytes were found to be capable of antigen presentation via major histocompatibility complex (MHC) II molecules, and these MHC II-antigen complexes are recognized by CD4⁺ cells.¹⁰⁴ Expression of the costimulatory molecule ICAM1 on the melanocyte cell surface correlated with T-cell responses to antigens presented by melanocytes.¹⁰⁴ Together, this may afford a partial explanation for vitiligo pathogenesis in that melanocytes with greater ICAM1 cell-surface expression experience increased T-cell recognition, which ultimately results in increased cytotoxic T-cell-mediated melanocyte destruction.

Ample support exists for the role of IFN- γ in vitiligo. For example, IFN- γ knockdown⁶⁸ and knockout^{28,105} mice experienced impaired depigmentation. The latter publication also provides insight into the detrimental effects observed following TNF- α inhibition in some patients. In IFN- γ knockout mice, depigmentation is still observed when Tregs are depleted, and IFN- γ knockout mice exhibited an increase in Tregs. This suggests that the mechanism by which IFN- γ can induce depigmentation is explained, at least in part, by suppressing Treg development.^{106,107}

Further supporting the importance of Tregs in vitiligo is the finding that upon adoptive transfer of Tregs, disease remission was measured in a mouse model of vitiligo.²⁸ Knowing that TNF- α activates Tregs *in vivo*³² and will support Treg development,^{30–32,108} it follows that depleting TNF- α can lead to decreased Treg production and activation, resulting in even less skin homing of Tregs in patients with vitiligo, which is problematic and relatively specific to vitiligo.³⁴ Inhibiting TNF- α may also reduce the number of available Tregs in the

skin of patients without vitiligo, permitting cytotoxic T lymphocytes the opportunity to exert an unchecked inflammatory response within the epidermis without suppression by Tregs, and tipping the scale in favour of depigmentation. This explanation may account for the unexpected worsening of pre-existing vitiligo and *de novo* vitiligo development observed in some patients treated with anti-TNF- α agents. It is then probable that such local Treg deficiency can be overcome by recruiting Tregs to the skin by means of topical CCL22 administration.¹⁰⁹ Once developed for human use, such treatment may be helpful in preventing the adverse development of vitiligo in patients treated with TNF- α inhibitors for other conditions.

Conclusions and future directions

Upon review, TNF- α appears to have both pro- and anti-inflammatory properties (Box 3), within the skin, possibly due to its ability to stimulate both cytotoxic T cells and Tregs. Although blocking TNF- α was effective in halting disease progression and even in promoting repigmentation in nearly every patient with progressive vitiligo, 18 of 5928 patients developed *de novo* vitiligo when treated for another autoimmune condition. Until such side-effects can be fully averted, anti-TNF- α treatment is unlikely to gain much traction in the treatment of vitiligo. An important observation is that halting depigmentation was not necessarily followed by promoting repigmentation. Indeed, in future clinical trials to treat vitiligo it will be of the utmost importance to consider both parameters when evaluating treatment efficacy, so that a positive outcome is not inadvertently overlooked. Given the complex nature of vitiligo development, it is possible that a combination of treatments will prove most efficacious for vitiligo treatment. To prevent vitiligo from developing as a side-effect of anti-TNF- α treatment in patients with other autoimmune conditions, it may be important simultaneously to promote Treg infiltration into the skin.

TNF- α inhibitors have actually provided significant benefit by halting disease that might otherwise be progressive. For this reason, failure to repigment should not necessarily be considered a treatment failure. Furthermore, etanercept should be considered the preferred anti-TNF- α agent to employ in patients at risk for developing vitiligo, in order to mitigate the risk of this adverse event during therapy for other conditions. In all, TNF- α inhibitors have offered benefit in patients with progressing vitiligo, while unexpected observations made during clinical trials for these agents have provided new insight into the differential involvement of TNF- α in autoimmune disease.

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