

TNF- α production in the skin

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Abstract Upregulation of TNF- α is a key early response to ultraviolet B (UVB) by keratinocytes (KCs), and represents an important component of the inflammatory cascade in skin. UVB irradiation induces TNF- α expression in both KCs and dermal fibroblasts, with TNF- α mRNA induction seen as early as 1.5 h after UVB. We previously reported that the effects are wavelength-specific: TNF- α expression and secretion are induced by UVB (290–320 nm), but not by UVA (320–400 nm). Moreover, we found that IL-1 α , a cytokine also present in irradiated skin, substantially and synergistically enhances the induction of TNF- α by UVB, and the induction of TNF- α by this combination of UVB with IL-1 α is mediated through increased TNF- α gene transcription. We investigated the molecular mechanism for UVB-induction of the TNF- α gene with a series of TNF- α promoter constructs, ranging from 1.2 kbp (from –1179 to +1 with respect to the TNF- α transcription initiation site) down to 0.1 kbp (–109 to +1), each driving expression of a CAT reporter. Our results showed a persistent nine to ten-fold increase of CAT activity in all TNF- α promoter/reporter constructs in response to UVB (30 mJ/cm²) exposure. These results indicate the presence of UVB-responsive *cis*-element(s) located between –109 and +1 of the TNF- α promoter, a region that contains a putative AP-1 site and a putative NF κ B site. UVB-induction was abolished when the TNF- α promoter was mutated by one base pair at

the AP-1 binding site. Cells treated with SP600125, an AP-1 inhibitor that inhibits JNK (c-Jun N-terminal kinase), also showed suppression of the 0.1 kbp TNF- α promoter/reporter construct. The authentic endogenous gene in untransfected cells was also blocked by the inhibitor. Electrophoretic Mobility Shift Assay indicated new complexes from UVB-treated nuclear extracts and anti-phospho-c-Jun, a regulatory component of the AP-1 transcription factor, creating a supershift indicating increased phosphorylation of c-Jun and hence higher AP-1 activity. Keratinocyte-derived TNF- α is a component of the early induction phase of the inflammatory cascade.

Keywords TNF- α · Ultraviolet light · Skin · Fibroblast · Keratinocyte

Abbreviations

KCs Keratinocytes
FBs Fibroblasts
UVB Ultraviolet B
TNF- α Tumor necrosis factor- α

Introduction

TNF- α has a myriad of pro-inflammatory effects on the skin. There is evidence that ultraviolet light induces early TNF- α release from keratinocytes (KCs) in both normal and skin of cutaneous lupus erythematosus (CLE). Recent data related to the molecular mechanisms of UVB induction of KC TNF- α will be reviewed. There is a polymorphism of TNF- α associated with subacute cutaneous LE, as well as other autoimmune diseases. There are synergistic interactions between pro-inflammatory cytokines produced in the skin and in synergy with irradiated KCs, leading to

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later augmentation of TNF- α production. Different cytokines in combination with UVB have specific effects on chemokine production by UV-irradiated KCs. Finally, the most recent data characterizing the mechanism of the molecular sensor responsible for UV-induced activation of the TNF- α promoter will be reviewed. Clearly there is a large interplay of proteins and other skin-derived molecules that lead to the induction of inflammation in irradiated skin.

TNF- α effects in skin

There is excellent evidence that TNF- α in the skin induces adhesion molecules and chemokines in the skin, leading to attachment of inflammatory cells to vessels, rolling, emigration, and eventually chemotaxis into the skin (See Fig. 1) [7, 17, 30]. In addition, UVB induces inducible nitric oxide synthase (iNOS) activity in dermal endothelial cells, which plays an important role in UVB-induced inflammation in human skin, through a TNF- α -dependent pathway, as well as apoptosis [27, 29, 36, 37]. These inflammatory cells secrete additional cytokines that form a positive feedback loop in further upregulating TNF- α and downstream TNF- α -induced chemokines, cytokines, and other pro-inflammatory pathways.

Evidence for TNF- α production by keratinocytes and fibroblasts

There is evidence that UV-irradiation of both normal and cutaneous LE skin induces TNF- α at the protein and mRNA level [3, 9, 14, 26]. In addition, irradiation of KCs by UVB induces TNF- α mRNA and protein production [8]. IL-1 α induces a synergistic induction of TNF- α by both UVB-irradiated KCs and fibroblasts (FBs), and UVB-irradiated FBs produce minimal TNF- α in the absence of IL-1 α [11, 33]. In the presence of IL-1 α , UVB-irradiated KCs begin to

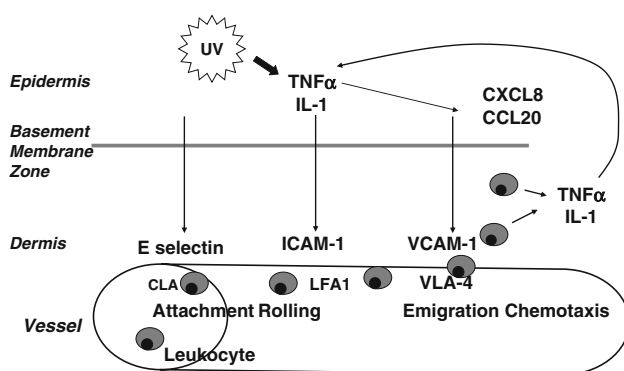


Fig. 1 Model of UV light/TNF- α effects in the skin. TNF- α induces adhesion molecules and specific chemokines in the skin

produce significant amounts of TNF- α protein between 3 and 4 h, with a peak by 24 h (Fig. 2a). There is evidence that induction of TNF- α by KCs and FBs is seen with UVB, but not UVA [33].

Molecular mechanisms of UVB induction of KC TNF- α

The TNF- α gene is present on chromosome 6, located between lymphotoxin- β and Lymphotoxin- α (Fig. 3). The exact molecular mechanism for UV-induced TNF- α secretion from skin cells has been unknown, although there has been evidence that transcriptional effects are important [4, 6]. Normal human epidermal KCs released TNF- α in the medium after a single dose of UVB [35]. Recent studies demonstrate that TNF- α mRNA is induced in UVB-irradiated KCs treated with IL-1 α by 1.5 h, with a peak at 8 h [4], followed by a steady decrease for 72 h (Fig. 2b). Nuclear run-off experiments demonstrate the UVB effect on TNF- α gene induction to be transcriptional in both macrophages

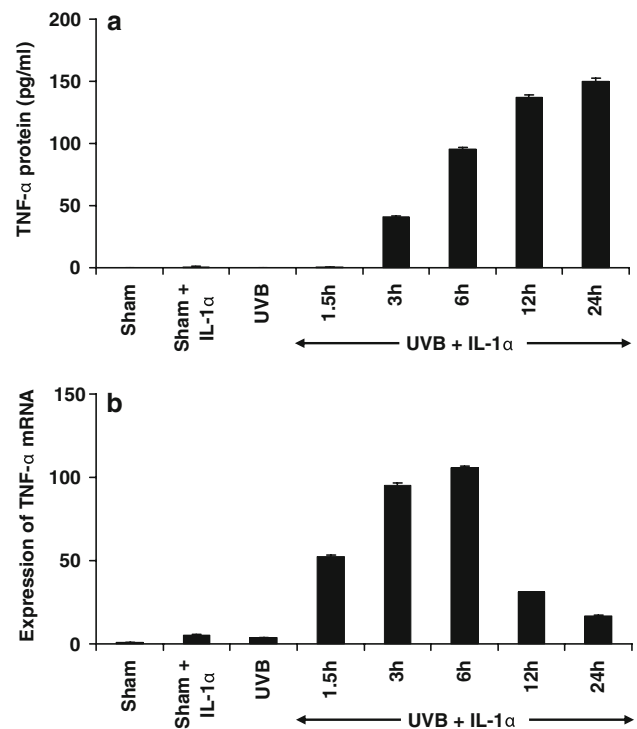


Fig. 2 Synergistic effects of UVB and IL-1 α on keratinocytes. Cells were treated with sham, sham + IL-1 α (10 ng/ml), UVB 30 mJ/cm² and UVB + IL-1 α . **a** Supernatant samples were collected at the indicated times and analyzed by ELISA. There is a synergistic increase of TNF- α protein in UVB + IL-1 α -treated keratinocytes. **b** RNA samples were collected at the indicated times and analyzed by real-time PCR. Results were normalized to cyclophilin A mRNA (housekeeping gene control) and presented as fold expression. IL-1 α synergistically increases TNF- α mRNA induction by UVB-irradiated neonatal keratinocytes

and KCs [4, 6]. There was no effect of IL-1 α on TNF- α mRNA half-life, suggesting that the increased TNF- α mRNA seen is predominantly due to transcriptional effects [4]. Previous studies have demonstrated that the UVB-signaling pathway in KCs is distinct from that seen with LPS, and mutations of each of the NF κ B sites did not affect the UVB responsiveness of TNF- α promoter constructs [6, 22].

TNF- α promoter polymorphisms

There is an association of the -308A TNF- α promoter polymorphism with one form of subacute cutaneous LE [34]. This polymorphism is associated with an extended HLA A*01, B*08, DRB1*0301 haplotype, and the role of the polymorphism in terms of its role in etiology or simply disease association is still unclear [13]. Some studies have demonstrated an increase in levels of transcription, but the effects are highly context specific in terms of the cells type and stimulus, as well as the features of the promoter construct utilized [1, 13, 15, 16, 24]. Based on the susceptibility to UVB-induced impairment of contact hypersensitivity, it was proposed that polymorphism of the TNF- α region attributed to deleterious effects of UVB radiation in both mice and human [23].

Skin cytokine effects on epidermal TNF- α

After inflammatory cells enter the skin, they secrete numerous cytokines that can further increase epidermal production of TNF- α . There is evidence that TNF- α and IL-1 α induce large increases of TNF- α mRNA expression in KCs [18]. However, clustering and internalization of TNF receptors may lessen the response to TNF- α protein over time, accounting for why persistent TNF- α in culture supernatant does not sustain TNF- α mRNA upregulation over time. Interferon- γ (IFN- γ) with IL-1 α , but not IFN- α can further augment TNF- α expression by KCs [20].

Cytokines in the skin have differential effects on chemokine induction

There is evidence that UV-irradiated KCs in the presence of interferon-gamma leads to CCL5 and CCL22 induction. On the other hand, UVB in the presence of TNF- α and IL-1 β induces CCL20, and CXCL8 in KCs. These chemokines are mainly regulated by IL-1 α and TNF- α , UVB-induced primary proinflammatory cytokines [21].

Molecular sensor of UV in keratinocytes

A number of transcription factors are induced by UV light. Two of these UV-regulated transcription factors [10], AP-1 and NF κ B, have known binding sites in the TNF- α promoter. There are, in fact, four NF κ B binding sites and one AP-1 binding site (Fig. 3) [6]. The nature of the photoreceptor that senses UV light in the TNF- α promoter is not known. LPS stimulates TNF- α gene transcription in macrophages via NF κ B, but site-directed mutations of the NF κ B elements in the TNF- α promoter failed to disrupt its induction by UV irradiation [6]. There are broad regions of transcriptional factor binding sites in the TNF- α promoter that have positive and negative response elements unrelated to UV [6]. The proximal 120 bp portion of the TNF- α promoter region possesses binding sites for NF- κ B, C/EBP (CCAAT-enhancer binding protein α) and AP-1 (c-Jun) transcription factors. Transient transfection of a 120-bp TNF- α promoter-reporter construct simultaneously transfected with a plasmid expressing the wild-type version of each of these transcription factors contributed to TNF- α promoter activation [19] (Fig. 4).

TNF- α promoter/CAT reporter deletion constructs have been made that demonstrate that the UV response element is contained between -109 and +1, in the very proximal aspect of the TNF- α promoter [4]. This proximal part of the hTNF- α promoter contains an AP-1 binding site

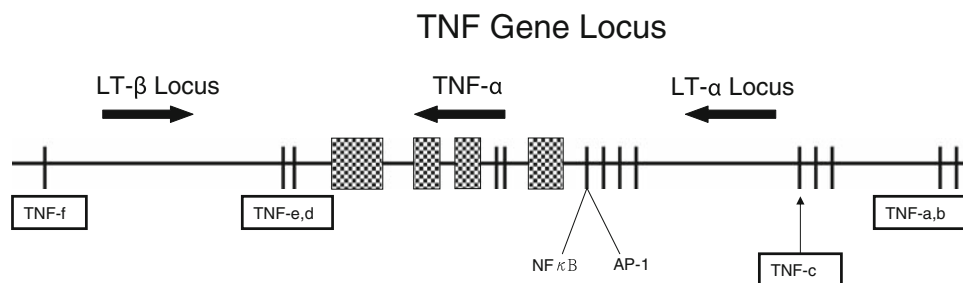


Fig. 3 Map of human TNF- α and flanking genes on chromosome 6. The human TNF- α gene is located on chromosome 6 and is flanked by lymphotoxin α and β genes. The position and direction of transcription

of the TNF- α , lymphotoxin α , and lymphotoxin β genes is shown by the *black thick arrows*. The exons are shown as *checked boxes*. The TNF-a, b, c, d, e, and f extended haplotype genetic markers are shown

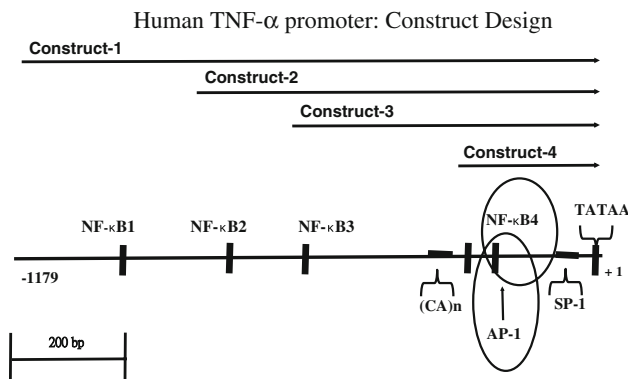


Fig. 4 Schematic diagram of human TNF- α promoter showing NF- κ B and AP-1 sites. The human TNF- α promoter has four NF- κ B and one AP-1 binding sites. Four different promoter/reporter constructs were designed containing different NF- κ B site. The construct designed from the proximal part of the TNF- α promoter contains one NF- κ B and AP-1 site, highlighted with circles

(TGAGCTTA) at -106 to -99 . AP-1 is a sequence-specific transcriptional activator, composed of the c-JUN/FOS heterodimer. In human skin, UV radiation elevates the expression of c-JUN, while FOS is constitutively expressed [31, 32]. Loss of the UVB response occurs by a G to T point mutation in the AP-1 binding site of the 1.2-kb TNF- α promoter [4]. Furthermore, inhibition of c-Jun N-terminal kinase, a stress-activated serine threonine protein kinase that phosphorylates c-Jun, results in a near-total block of the UVB induction of several TNF- α /CAT reporter promoter fragments when they are transfected into human KCs. This includes a small fragment of the proximal promoter that contains the AP-1 binding site and just one of the four NF- κ B binding site [4]. In addition, nuclear proteins were isolated from irradiated KCs and incubated with 32 P-labeled oligonucleotide containing the AP-1 binding site of the human TNF- α promoter. This complex was then separated on a non-denatured polyacrylamide gel and demonstrated a supershift in mobility on EMSA when antibody against phosphorylated c-Jun was added. This also suggests a role for AP-1 binding to the TNF- α promoter as part of the UV response.

Activation of c-Jun N-terminal kinase is a KC specific UVB response, which in turn activates Elk-1 and AP-1 [2]. Studies in our lab showed AP-1 is a major regulator of TNF- α upregulation in response to UVB exposure [5]. TNF- α in turn has been shown to activate the NF- κ B pathway via IKK- β the active subunit of IKK.

Suppression of TNF- α in cutaneous LE

It is clear that TNF- α has a pro-inflammatory role in the skin, as discussed above. However, blockade of TNF- α is

complex in terms of cutaneous LE. Patients treated with anti-TNF- α agents show increased expression of interferon-regulated genes [25]. Suppression of TNF- α can induce skin disease. In addition, there is evidence that TNF- α has regulatory effects on B cell differentiation [12].

Conclusion

TNF- α plays an important pro-inflammatory role in the skin, both due to direct effects of ultraviolet light and to indirect effects of inflammatory cells that chemotax to the skin. It is clear that UV- and inflammatory cell-derived cytokines further enhance TNF- α gene transcription in human KCs and FBs. Recent studies suggest that AP-1 plays a critical role in increasing TNF- α transcription in UVB-irradiated KCs. IL-1 further augments TNF- α transcription through mechanisms independent of AP-1. Blockade of TNF- α in lupus has complex effects because of augmentation of interferon-regulated genes.

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Conflict of interest statement The authors have no potential conflict of interest.

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