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VEGF-Trap decreases CD4⁺ T cells and Th17 cytokines improving psoriasis-like skin inflammation in KC-Tie2 mice

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The reddened appearance of lesional psoriasis skin reflects increases in angiogenesis and although this increase is unlikely to be the principle cause of psoriasis it may provide new therapeutic targets. VEGF is a potent mediator of angiogenesis [1], elevated VEGF is found in lesional skin and sera of psoriasis patients and correlates with disease severity and several VEGF genetic polymorphisms have been identified associated with early onset psoriasis.

VEGF contributions to psoriasis pathogenesis are multiple and include increasing cutaneous vascular permeability and inflammation, promotion of cutaneous leukocyte infiltration, eliciting pro-inflammatory T cell differentiation, and activating and inducing keratinocyte (KC) proliferation. Thus, VEGF serves autocrine and paracrine functions in psoriasis, promoting pro-inflammatory feedback loops between KCs, endothelial cells, infiltrating T cells and monocytes which together exacerbate cell-mediated immune inflammatory reactions.

VEGF importance in psoriasis is further evidenced by the development of a psoriasiform phenotype in KC-VEGF overexpressing mice, where skin phenotypes similar to human psoriasis develop following oxazolone [2] or TPA [3] treatment or tape stripping in young and spontaneously in older mice [4]. In each model, KC-derived VEGF and angiogenesis increased prior to lesion development, and in lesional skin, immunocyte infiltrate included T cells and macrophages [2, 4]. Targeted VEGF-inhibition improved the skin disease, however dermal T cells remained present in one study [4], and VEGFR1 or VEGFR2-specific inhibition individually failed to modify the inflammatory response, but together resolved the psoriasiform phenotype [2].

Despite recent reports of patients experiencing psoriasis remission during bevacizumab treatment for colon cancer [5] and following sorafenib treatment for renal cell carcinoma [6], targeting VEGF in psoriasis has received minimal attention. Using the KC-Tie2 psoriasiform mouse model, we investigated the efficacy of anti-VEGF treatment in skin inflammation. KC-Tie2 mice spontaneously develop skin disease that phenocopies human psoriasis, including increases in angiogenesis, VEGF (~3-fold; $P < 0.02$), psoriasis-related pro-inflammatory leukocytes and cytokines, and disease resolution following CsA treatment [7].

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Conflicts of Interest: None.

Adult KC-Tie2 mice and littermate controls were treated with subcutaneous VEGF-Trap or control FC protein (25mg/kg; n=8/group; Regeneron Pharmaceuticals, Tarrytown, NY) 2x/week for 4 weeks similar to what has previously been done [4]. VEGF-Trap is a soluble fusion protein that binds VEGF with high affinity, has an extended *in vivo* half-life in mice and consists of portions of human VEGFR1/R2 and the Fc domain of human IgG1. All animal protocols were approved by the Case Western Reserve University IACUC.

Skin was examined histologically using H&E, immunohistochemistry and at the RNA and protein levels as previously described [7, 8].

All data are presented as mean \pm SEM. Data were tested for normality and statistical significance calculated using either a Student t test or a Mann–Whitney U test, as appropriate. Significance was defined as $P < 0.05$.

VEGF-Trap significantly reduced dermal angiogenesis to control levels in KC-Tie2 mice ($P=0.04$ vs KC-Tie2+FC protein) with concomitant reductions in acanthosis (47%; $P=0.03$ vs KC-Tie2+FC protein) and decreases in innate defense genes S100A8/A9 and DefB3 (all $P<0.03$; Fig. 1). Despite significant decreases in cutaneous CD11b⁺, CD11c⁺ and F4/80⁺ cells in VEGF-Trap treated KC-Tie2 mice ($P<0.001$ vs KC-Tie2+FC protein), levels remained significantly increased compared to control littermates ($P<0.04$). Myeloid cell-derived cytokines, TNF α , IL-1 α , IL-6 and IL-12 remained elevated following VEGF-Trap treatment whereas IL-23 expression returned to control mouse levels ($P=0.02$ vs KC-Tie2+FC protein; Fig. 2). Interestingly, CD4⁺ T cells decreased (63%; $P=0.001$) in VEGF-Trap treated KC-Tie2 mouse skin, accompanied by significant reductions in IL-17A, IL-17F and IL-22 (all $P<0.007$; Fig. 2), whereas CD8⁺ T cells remained elevated along with IFN γ levels.

Together, these data provide *in vivo* evidence for efficacy of VEGF-Trap treatment for psoriasis and suggest disease improvement may occur via VEGF-mediated effects on the IL-23/Th17 axis of inflammation. These findings are consistent with VEGF inhibition in two prior genetic VEGF-overexpressing models of skin inflammation [2, 4], as well as recent evidence showing VEGF-inhibition (using G6-31) efficacy in the KC-specific *JunB:c-Jun* knockout mouse [9]. However, we provide mechanistic insight into how VEGF-mediated inhibition occurs, identifying reductions in Th17 cell presence and activation (via decreased CD4⁺ T cells and IL-17A/F and IL-22) in VEGF-Trap treated mice; possibly as a result of decreased levels of IL-23 but not IL-12. This observation validates and expands upon a prior report demonstrating decreased infiltrating CD3/4⁺ T cells and reductions in IL-22 [9]. Although significant decreases in myeloid cell infiltrates were observed in VEGF-Trap treated KC-Tie2 mouse skin, and decreased IL-23, other myeloid-cell derived cytokines, including TNF α , IL-12, IL-1 α and IL-6 remained elevated, potentially explaining the significant improvement, but not complete reversal of acanthosis, Th1 presence (and increased IFN γ) and skin disease severity. This is reminiscent of prior work where significant improvement in skin disease severity, but not complete reversal, was found in KC-Tie2 mice treated with antibodies targeting TNF α [8]. Interestingly, recent attempts blocking both VEGF and TNF α , using a novel fusion protein targeting both molecules, called Valpha [3] has proven highly effective at reversing/preventing TPA-induced acanthosis and dermal lymph/angiogenesis in KC-VEGF animals at levels greater than either TNF α (Enbrel) or VEGF (VEGF-Trap) inhibition alone. VEGF-Trap also inhibits PIGF-signaling events [4], and PIGF expression levels were also elevated in KC-Tie2 mouse skin (21.66 ± 6.6 pg/ml KC-Tie2 vs 8.4 ± 2.7 pg/ml controls; $P=0.002$), thus the VEGF-Trap effects reported here likely reflect VEGF- and PIGF-inhibition. Interestingly, our observation that CD8⁺ T cells remain elevated along with IFN γ levels in KC-Tie2 mice treated with VEGF-Trap suggests disease severity can improve despite sustained Th1

activity if Th17 cell depletion is sufficient, and is consistent with recent clinical trials showing excellent efficacy of IL-17 inhibition in psoriasis patients.

In conclusion, we demonstrate VEGF-Trap treatment improves chronic skin inflammation in KC-Tie2 mice and that disease resolution occurs in an IL-23/Th17 mediated manner. Although systemic VEGF inhibition is unlikely to become a frontline target for psoriasis drug development due in part to its potential side effects; our data provide proof of concept support for targeting this molecule and its multi-cellular functions in psoriatic skin. Targeting VEGF topically may provide an effective new approach for treating mild-to-moderate psoriasis skin lesions by inhibiting angiogenesis and reducing leukocyte infiltration and KC proliferation.

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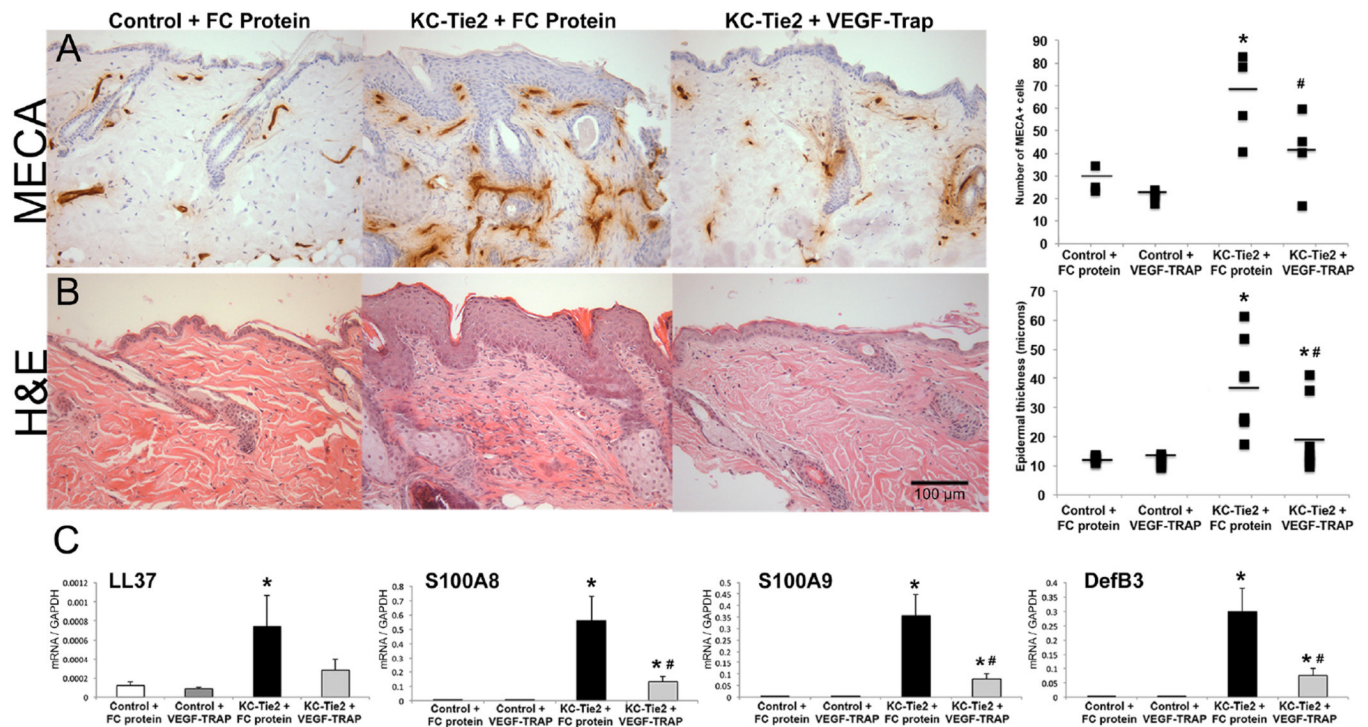


Figure 1. VEGF-Trap treated KC-Tie2 mice have decreased angiogenesis, acanthosis and innate defense gene expression

Representative back skin sections from control and KC-Tie2 mice treated with control FC protein (25mg/kg) or VEGF-Trap (25mg/kg) immunostained using the endothelial cell-specific antibody (MECA) (A) or stained with H&E (B). Quantification of angiogenesis (MECA⁺ blood vessel number) (A), epidermal thickness (B) and qRT-PCR gene expression analyses of psoriasis-related innate defense molecules (C) are presented (n=8/group; mean ± SEM). * P<0.05 vs Control + FC protein; # P<0.05 vs KC-Tie2 + FC protein. Scale bar = 100µm.

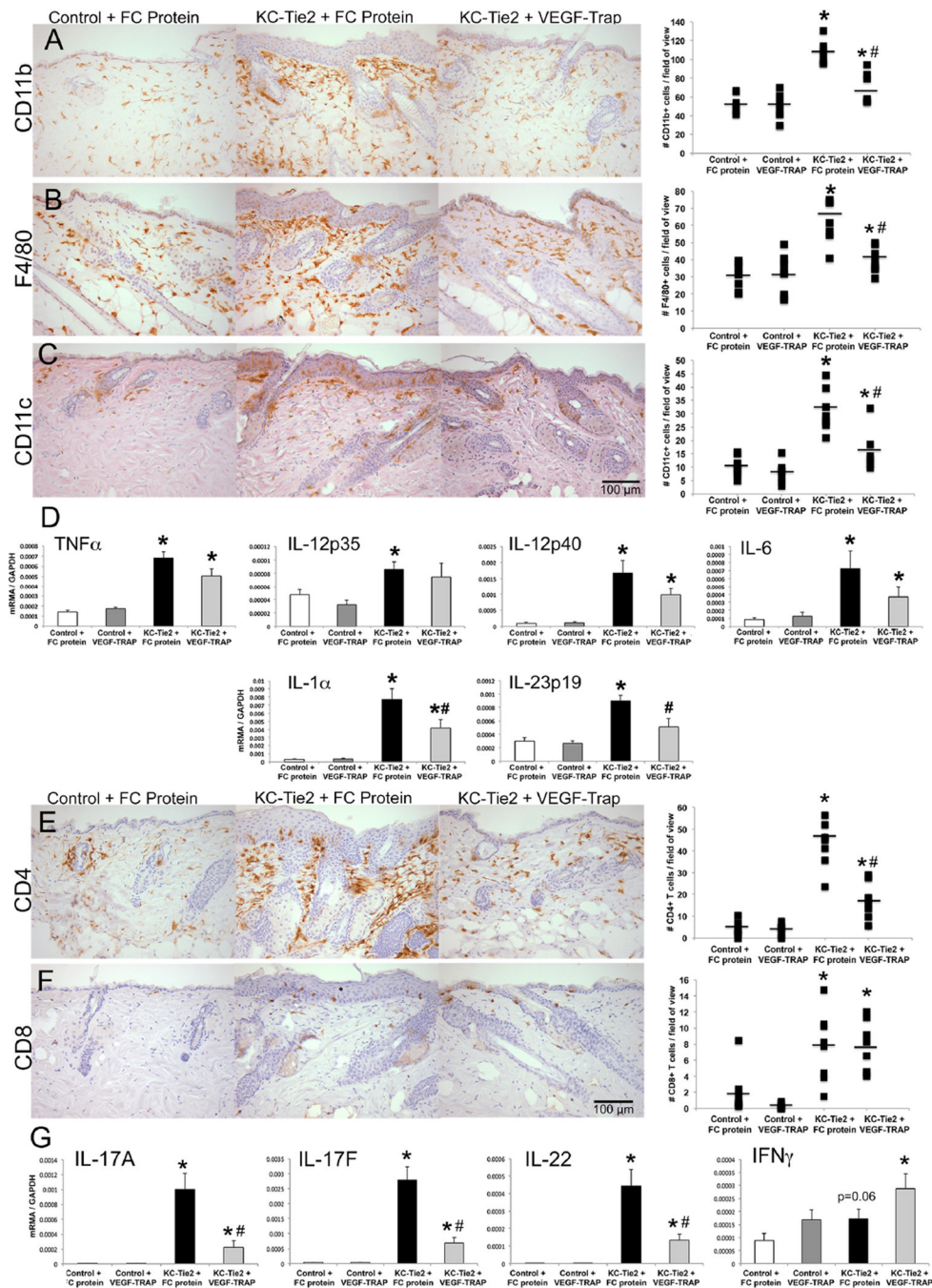


Figure 2. VEGF-Trap treated KC-Tie2 mice have fewer cutaneous infiltrating myeloid cells, CD4⁺ T cells and reduced IL-23 and Th17 cytokines
 Representative back skin sections from control and KC-Tie2 mice treated with control FC protein (25mg/kg) or VEGF-Trap (25mg/kg) immunostained using antibodies targeting CD11b (A), F4/80 (B), CD11c (C), CD4 (E) and CD8 (F) antigen. Quantification of skin infiltrating myeloid cells (A-C), T cells (E, F) and qRT-PCR gene expression analyses of myeloid-derived cytokines (D) and T cell derived cytokines (G) are presented (n=8/group; mean \pm SEM). * P<0.05 vs Control + FC protein; # P<0.05 vs KC-Tie2 + FC protein. Scale bar = 100 μ m.