Involvement of IL-9 in Th17-Associated Inflammation and Angiogenesis of Psoriasis

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Abstract

It is thought that a Th1/Th17-weighted immune response plays a predominant role in the pathogenesis of psoriasis. Our findings now indicate a link between IL-9, a Th2 and Th9 cytokine, and Th17 pathway in psoriasis. In K5.hTGF-β1 transgenic mice, exhibiting a psoriasis-like phenotype, we found increased IL-9R and IL-9 expression in the skin and intradermal IL-9 injection induced Th17-related inflammation. IL-9 also promoted angiogenesis and VEGF and CD31 overexpression in mice in vivo and increased tube formation of human endothelial cells in vitro. Injecting anti-IL-9 antibody into K5.hTGF-β1 transgenic mice not only diminished inflammation (including skin infiltration by T cells, monocytes/macrophages, and mast cells) and angiogenesis but also delayed the psoriasis-like skin phenotype. Notably, injection of anti-psoriatic acting anti-IL-17 antibody reduced skin IL-9 mRNA and serum IL-9 protein levels in K5.hTGF-β1 transgenic mice and prevented IL-9-induced epidermal hyperplasia and inflammation of the skin of wild type mice. In addition, we observed that IL-9R expression in lesional skin from psoriasis patients was markedly higher than in healthy skin from control subjects. Moreover, IL-9 significantly enhanced IL-17A production by cultured human peripheral blood mononuclear cells or CD4+ T cells, especially in psoriasis patients. Thus, IL-9 may play a role in the development of psoriatic lesions through Th17-associated inflammation and angiogenesis.

Introduction

Interleukin (IL)-9, a member of the IL-2 cytokine family [1], is secreted by naïve CD4+ T cells in response to TGF-β and IL-4 (Th9 pathway) [2]. However, IL-9 is also produced by activated Th2 lymphocytes and is involved in Th2-associated diseases [3–6]. Moreover, IL-9 is a growth factor for mast cells and T cells that help facilitate the Th9 immune response of allergic inflammatory diseases including asthma [3–4,6–8]. The differentiation of Th9 and Th2 cells seems to be regulated by different transcription factors depending upon the cytokine environment [9–11]. Intriguingly, IL-9 can also induce Th17 cells to differentiate and mediate autoimmune and inflammatory diseases [3,12–14]. IL-9 is also produced by Th17 cells, which secrete mainly IL-17A and IL-17F [2,11]. When administered alone or with IL-6 and TGF-β1, IL-9 greatly enhances the production of IL-17 from Th17 cells in vitro [2,15]. Together these observations and the location of IL-9 gene on chromosome 5 (5q31.1) [3], a psoriasis susceptibility region (5q31.1-q33.1) [16] (Figure S1), prompted us to investigate the pathogenic role of IL-9 in psoriasis.

Psoriasis is one of the most common chronic inflammatory skin disorders characterized by hyperplastic epidermis with hyperkeratosis, infiltration of the dermis with inflammatory cells including T cells, macrophages, and mast cells, and increased angiogenesis with an underlying Th1/Th17-dominated immune response [17–20]. To address the potential role of IL-9 in psoriasis we utilized both K5.hTGF-β1 transgenic mice, which exhibit a phenotype similar to human psoriasis [21–27], and wild type (WT) mice. In addition, we studied IL-9R expression in psoriatic skin lesions and on CD4+ T cells and the effect of IL-9 on IL-17A production in cultured human peripheral blood mononuclear cells or CD4+ T cells from psoriasis patients.

Materials and Methods

Human Subjects

Blood was collected from patients with moderate to severe chronic plaque type psoriasis (i.e., body size area involved >10%) and healthy control volunteers at the Photodermatology Research Unit, Department of Dermatology, Medical University of Graz, Austria (clinical study protocol approval no. 18–116 ex 06/07, 2013).
Ethics Committee of the Medical University of Graz. Written consent was obtained from all patients and volunteers enrolled in the study. PBMCs were isolated by using Lymphoprep™ (Axis-Shield, Heidelberg, Germany) and used for CD4+ T cell isolation or fluorescence-activated cell sorting (FACS) analysis. Skin samples were taken from the psoriasis patients or healthy control subjects for hematoxylin-eosin (HE) and immunohistochemical staining and analysis.

Mice

Hsd:ICR/CD-1 R WT and K5.hTGF-β1 transgenic mice (on an Hsd:ICR/CD-1 R background) were used, as previously described [22,23]. The mice were bred at the University Medical Center, Göttingen, Germany, from which they were shipped to the Medical University of Graz, where all experiments were performed. The mice were housed in the animal facility of the Center for Medical Research, Medical University of Graz, and maintained under condition of alternating 12-h light and dark cycles, controlled temperature, and controlled humidity in facilities approved by the Austrian Government. Water and food were provided ad libitum. All procedures to which the mice were subjected were approved by the Austrian Government, Federal Ministry for Science and Research, through protocol no. BMWF-2011. Mice were 8–10 weeks old at the start of an experiment.

Skin Disease Severity Score

A specific disease severity score was used to rate the macroscopic appearance of mouse skin. In brief, each of three symptoms (erythema, infiltration, and scaling) was scored separately as 0 (not present), 1 (mild), 2 (moderate), or 3 (severe), and then the scores were summed, as previously described [22,23]. The highest possible score was 9.

Murine Tissue Collection

Mice were sacrificed 48 hours after the final antibody injection (at the end of a 4-week treatment period) or 24 hours after the last IL-9 injection and blood, spleen, and skin samples were collected. Approximately 1 cm² of central dorsal skin per mouse was excised, fixed immediately in 4% buffered formaldehyde, processed routinely, and embedded in paraffin. In addition, fresh skin tissue was fixed immediately in 4% buffered formaldehyde, processed and embedded in paraffin. The reactions were run on an Applied Biosystems 7900HT system in RT² SYBR Green/ROX qPCR Master Mix (Super Array Biosciences Corporation, Frederik, MD). The reactions were run on an Applied Biosystems 7900HT system in RT² SYBR Green/ROX qPCR Master Mix (Super Array Biosciences Corporation). The delta-delta Ct method was used to normalize transcript levels to GAPDH levels and to calculate fold-change from transcript levels in WT control skin samples.

In vitro Angiogenesis Assay

IL-9-dependent tube formation in HMVEC cells was assayed using the In Vitro Angiogenesis Assay Kit (Millipore, Billerica, MA). Total 2×10⁵ cells were seeded in ECM medium with or without 100 ng/mL human rIL-9 (eBiosciences) and then cells placed on top of ECMMatrix gels in 48-well plates and incubated for 48 hours. Tube formation was assayed after 24 and 48 hours.

Injection of rIL-9 into Mouse Skin

Murine recombinant IL-9 (eBiosciences) (500 ng) or PBS vehicle control was injected into the dorsal skin of WT or K5.hTGF-β1 transgenic mice daily for 4 days. Twenty-four hours after the last injection, mice were sacrificed and their dorsal skin was collected.

Isolation and Analysis of CD4+ T cells

For isolation and analysis of CD4+ T cells from human PBMCs, non-CD4+ T cells were depleted by magnetic cell sorting (MACS®, Miltenyi Biotec, Bergisch Gladbach, Germany). Isolated CD4+ T cells (1×10⁶) were cultured in 48- or 96-well plates (Nunc, Roskilde, Denmark) in the presence or absence of different combinations of rIL-6 (30 ng/mL), rTGF-β1 (3 ng/mL), and rIL-9 (20 ng/mL) for 4 days in RPMI medium (Sigma, St. Louis, MO, USA) supplemented with 10% FCS (v/v), penicillin (100 IU/mL), streptomycin (100 μg/mL), and L-glutamate (2 μM) (PAA Laboratories, Pasching, Austria); then, leukocyte-activating cocktail (BD Pharmingen) was added to the culture media for the last 4 hours. Cells were activated using plate-bound anti-CD3 (5 μg/mL) and soluble anti-CD28 (2.5 μg/mL). For intracellular staining of cytokines, cells were first stained for CD4 surface antigen, treated with Fix/Per (Fix/Per buffer; eBiosciences), and then further stained for IL-17A, according to the manufacturer’s instructions. The stained cells were then subjected to FACS analysis on a FACSCalibur flow cytometer and the data were analyzed with Flow Jo software (Tree Star Inc., Ashland, OR).

ELISA and ELISpot Assay

Human or mouse IL-9 and IL-17 enzyme-linked immunosorbent assay (ELISA) kits (eBiosciences) were used to quantify IL-9 and IL-17 protein levels in serum or culture supernatants of PBMCs or CD4+ T cells, according to the manufacturer’s instructions. A human IFN-γ/IL-17 dual-color ELISpot assay kit (R&D Systems) was used to quantify IFN-γ-secreting, IL-17-secreting, or IFN-γ/IL-17-co-secreting CD4+ T cells.

Real-time RT-PCR Analysis

RNA was isolated from mouse dorsal skin with a QIAGEN fibrous mini kit (QIAGEN, Valencia, CA). RNA was reverse-transcribed with a First Strand cDNA Synthesis kit (Roche) and then subjected to quantitative RT-PCR with pretested primers for STAT3, IL-17A, and IFN-gamma (Super Array Biosciences Corporation, Frederik, MD). The reactions were run on an Applied Biosystems 7500HT system in RT² SYBR Green/ROX qPCR Master Mix (Super Array Biosciences Corporation). The delta-delta Ct method was used to normalize transcript levels to GAPDH levels and to calculate fold-change from transcript levels in WT control skin samples.
Neutralization of in vivo Bioactivity of IL-9 and IL-17

Anti-IL-9 (10 mg/kg) antibody, anti-IL-17 (10 mg/kg) antibody, or isotype IgG antibody (control) was injected intraperitoneally in K5.hTGF-β1 transgenic mice twice a week for 4 weeks. This was done to assess the neutralizing effects of the antibodies on the in vivo bioactivity of IL-9 and IL-17.

Statistical Analysis

The number of neutrophils did not significantly differ between the different treatment groups upon IL-9 injection (data not shown). On the other hand, treatment of the transgenic mice with anti-IL-17 antibody twice a week for 4 weeks inhibited the psoriatic-like skin phenotype and downregulated IL-9 mRNA in skin and protein levels in serum (data not shown).

To gain further insight of IL-9’s role in psoriasis, we examined the functional role of this cytokine by giving intradermal injection of IL-9 into the back skin of WT mice. Injecting IL-9 once daily for 4 days induced skin inflammation by increasing epidermal hyperplasia (Figure 1A,B) and skin infiltration by CD3+ T cells, CD68+ monocytes/macrophages, and mast cells (Figure 1D). The number of neutrophils did not significantly differ between the different treatment groups upon IL-9 injection (data not shown). On the other hand, treatment of the transgenic mice with anti-IL-17 antibody twice a week for 4 weeks inhibited the psoriatic-like skin phenotype and downregulated IL-9 mRNA in skin and protein levels in serum (data not shown).

Results

IL-9 Promotes Skin Inflammation in Mice

First, we evaluated IL-9 levels in K5.hTGF-β1 transgenic mice and found that IL-9 protein and mRNA expression and IL-9R protein levels were higher in the skin of the transgenic mice than in the skin of WT mice (Figure 1A,B). Injecting IL-9 daily for 4 days into the skin of K5.hTGF-β1 transgenic mice enhanced epidermal hyperplasia (Figure 1C) and skin infiltration by CD3+ T cells, CD68+ monocytes/macrophages, and mast cells (Figure 1D). The number of neutrophils did not significantly differ between the different treatment groups upon IL-9 injection (data not shown). On the other hand, treatment of the transgenic mice with anti-IL-17 antibody twice a week for 4 weeks inhibited the psoriatic-like skin phenotype and downregulated IL-9 mRNA in skin and protein levels in serum (data not shown).

To gain further insight of IL-9’s role in psoriasis, we examined the functional role of this cytokine by giving intradermal injection of IL-9 into the back skin of WT mice. Injecting IL-9 once daily for 4 days induced skin inflammation by increasing epidermal hyperplasia (Figure 2A,B) and skin infiltration by CD3+ T cells, CD68+ monocytes/macrophages, and mast cells (Figure 2C). We further hypothesized that IL-17 may mediate skin inflammation after IL-9 injection, given that in humans IL-9 increased Th17 differentiation [3]. Blocking IL-17 before IL-9 injection resulted in complete reversal of skin inflammation and epidermal hyperplasia (Figure 2D,E).

IL-9 Induces the Th17 Pathway in Mice

To examine the role of Th17-cell mediated inflammation in the effects of IL-9, we analyzed the IL-9-injected skin from WT and K5.hTGF-β1 mice. We initially found and confirmed here that the transgenic mice had higher epidermal protein and total skin mRNA levels of STAT3 (Figure 3A–C) as well as skin IL-17 mRNA (Figure 3A) than the WT mice [22,23]. Recombinant IL-9 treatment further enhanced the expression of STAT3 proteins and mRNA of STAT3 and IL-17A in both transgenic and WT mice (Figure 3A–C). However, when we examined the effect of IL-9 on the pro-psoriatic cytokine IFN-γ [20], we found that IL-9 had no effect on IFN-γ mRNA expression in the skin of the transgenic mice (Figure 3A).

IL-9 Induces Angiogenesis in vitro and in vivo

Increased blood vessel formation and morphological and functional changes of microvessels are typical features of chronic inflammatory disorders, including psoriasis [24]. To evaluate the effect of IL-9 on blood vessel formation or angiogenesis, we injected IL-9 to the skin of WT and K5.hTGF-β1 transgenic mice. We observed that IL-9 increased the presence of angiogenic markers VEGF and CD31 in both WT and K5.hTGF-β1 transgenic mice (Figure 4A–C) as revealed by immunohistochemistry of the IL-9-injected skin. Next, we performed an in vitro angiogenesis assay with human dermal microvascular endothelial cells (HDMECs) to confirm the direct effect of IL-9 on blood vessel formation. We found that IL-9 significantly increased tube formation in HDMECs from 9.0±2.7 (baseline) to 29.2±0.8% (p<0.0001), as measured by number of vascular joints or bifurcations (Figure 4D,E).
IL-9 Neutralization Alters the Psoriatic-like Skin Inflammation and Inhibits Angiogenesis in K5.hTGF-β1 Transgenic mice

IL-9 neutralization has been effective in other models of autoimmune disease, including experimental autoimmune encephalitis (EAE). Anti-IL-9 treatment not only attenuated the diseases but also altered Th17 development in EAE [12,14]. In sight of this, we neutralized the bioactivity of IL-9 in K5.hTGF-β1 transgenic mice by injecting anti-IL-9 antibody (10 mg/kg) twice a week for 4 weeks. We observed that anti-IL-9 treatment led to marked alleviation of the psoriatic phenotype in K5.hTGF-β1 transgenic mice (Figure 5A,B). The effect on macroscopic phenotype alterations was greatest at week 2, when the mean skin severity score in anti-IL-9-treated transgenic mice was 37% lower than in IgG-treated control mice (ie, 2.2±0.3 vs. 3.5±0.6; p<0.01) (Figure 5A). Anti-IL-9 treatment in transgenic mice

Figure 1. IL-9 accelerates psoriasis-like inflammation in K5.hTGF-β1 transgenic mice. (A) Representative photomicrographs of immunohistochemical staining of IL-9 and IL-9R in the dorsal skin of K5.hTGF-β1 transgenic mice (Scale bar 200 μm for WT and 50 and 100 μm for K5.hTGF-β1 epidermis and dermis, respectively). (B) Real time PCR analysis for IL-9 in the dorsal skin of WT and K5.hTGF-β1 transgenic mice (n=7 mice per group). (C) K5.hTGF-β1 transgenic mice were injected intradermally for 4 days with 500 ng of IL-9 or vehicle (PBS) and skin samples were collected 24 hours after the last IL-9 injection (C,D). Representative photomicrographs of HE-stained paraffin-embedded skin sections (Scale bar 200 μm). Histological quantification of mean epidermal thickness (n=5 mice per group). (D) Dermal infiltration by CD3+ T cells, CD68+ monocytes/macrophages, and mast cells in the dorsal skin of WT mice (n=5 mice per group). Data shown represent mean numbers of cells per >200 microscopic field. Error bars represent SEM. *, p<0.05 (unpaired t-test). Similar results were obtained in two independent experiments. doi:10.1371/journal.pone.0051752.g001
not only delayed the onset of macroscopic disease progression but also reduced histological changes, including epidermal hyperplasia and skin infiltration by CD3+ T cells, CD68+ monocytes/macrophages, and mast cells (Figure 5B, C and Figure S2). The number of neutrophils did not significantly differ between the different groups upon anti-IL-9 treatment (data not shown).

To assess the immune response in skin, qRT-PCR on skin samples from K5.hTGF-β1 transgenic mice either treated with control IgG or anti-IL-9 antibody was performed. Anti-IL-9 treatment reduced the mRNA expression of IL-17A and STAT3 in the transgenic mice (Figure 5D). In addition, there was a trend that anti-IL-9 treatment lowered IFN-γ mRNA expression in the skin.

We found that injecting IL-9 increased angiogenesis in K5.hTGF-β1 transgenic mice and Zibert et al. [24] showed that halting angiogenesis in K5.hTGF-β1 transgenic mice alleviated psoriasis-like skin inflammation. Thus, we analyzed blood vessel formation in skin and soft tissue after anti-IL-9 or control IgG antibody injection after 4 weeks of treatment. Anti-IL-9 treatment reduced macroscopic blood vessels in the skin of K5.hTGF-β1

Figure 2. IL-9 induces inflammation in WT mice. WT mice were injected intradermally once daily for 4 days with 500 ng of recombinant IL-9 or vehicle (PBS) and skin samples were collected 24 hours after the last IL-9 injection. (A) Representative photomicrographs of HE-stained paraffin-embedded skin sections. (B) Histological quantification of mean epidermal thickness (n = 3 mice per group). (C) Dermal infiltration by CD3+ T cells, CD68+ monocytes/macrophages, and mast cells in the dorsal skin of WT mice (n = 3 mice per group). Data shown represent mean numbers of cells per x200 microscopic field. (D) Representative photomicrographs of HE-stained paraffin-embedded skin sections of WT mice injected i.p. with either anti-IL-17 antibody or IgG isotype control antibody immediately before the first intradermal injection of 500 ng IL-9. (Scale bar 200 μm). (E) Histological quantification of mean epidermal thickness or semi-quantitative rating of total dermal inflammatory cell infiltrate (0, no; 1, mild; 2, moderate; and 3, severe) in each experimental group (n = 4 mice per group). Error bars represent SEM. *, p<0.05; **, p<0.01; ***, p<0.001. (unpaired t-test). Similar results were obtained in three independent experiments. doi:10.1371/journal.pone.0051752.g002
transgenic mice (Figure S2). Moreover, IL-9 neutralization resulted in marked reduction in the expression of VEGF and CD31 as compared to treatment with control IgG in K5.hTGF-β1 transgenic mice on the microscopic level (Figure 5E,F).

IL-9 Promotes IL-17A Production in Human Psoriasis

To tease out the role of IL-9 in human psoriasis, we first analyzed and compared IL-9 receptor (IL-9R) expression by immunohistochemistry. We found increased IL-9R expression in scattered cells of the dermis, particularly at the dermal-epidermal junction, and within the basal layers of the epidermis in psoriatic patients compared to normal subjects (Figure 6A,B). We also detected significantly higher IL-9 levels in culture supernatant of activated CD4+ T cells (10.7 ± 0.6 pg/ml vs. ND) (Figure 6C). Moreover, addition of IL-9 alone or together with IL-6 and TGF-β1 enhanced production of IL-17A by cultured and activated human psoriatic CD4+ T cells after polyclonal stimulation (Figure 6F). Co-culture of CD4+ T cells with IL-9, TGF-β1, and IL-6 also significantly increased IL-17A+ CD4+ T cell numbers (Figure S3). Notably, IL-9-induced secretion of IL-17A and increase in IL-17A+CD4+ T cell numbers was greater for psoriasis patients than for normal healthy controls (Figure S3).

We also checked the effect of IL-9 on IL-17 and IFN-γ secretion by cultured CD4+ T cells isolated from human psoriatic PBMCs by ELISPOT and found increased numbers of IL-17 secreting cells but no change in the numbers of cells secreting IFN-γ or IFN-γ/IL-17 (Figure 6E).

Discussion

Our findings indicate a link between IL-9, a Th2 and Th9 cytokine, and Th17 pathway in psoriasis. We found markedly higher expression of IL-9R in psoriatic skin lesions. In addition, we observed that ex vivo IL-9 stimulated the production of IL-17 by peripheral blood mononuclear cells or CD4+ T cells, especially in cells isolated from individuals with psoriasis (Figure 6A–D). Accordingly, we also found that addition of IL-9 together with IL-6 and TGF-β1 increased the production of IL-17A from cultured and activated CD4+ T cells (Figure 6F). Consistent with these data, Th17 cells are known to express the receptor for IL-9 [3,11,15]. Although, our experiments did not distinguish effects of IL-9 on the de novo production of Th17 cells from naive cells vs. effects on effector/memory cells, earlier reports suggest that IL-9 can contribute directly to Th17 differentiations [15], and our data...
indicate that IL-9 may have such activity in the context of psoriasis. In addition, ELISPOT assays using cells from individuals with psoriasis showed that IL-9 had no ex vivo effects on numbers of IFN-γ secreting CD4+ T cells, suggesting that IL-9 makes no contribution on the Th1 component of the disease (Figure 6E). This observation is also consistent with reports of IL-9’s inhibitory or null effect on IFN-γ [3,12,28]. Consistent with the work of others [29] we are finding that most psoriasis patients have significantly elevated IL-17 levels in the serum but intriguingly only approximately one third of them have measurable IL-9 levels (Wolf et al., unpublished data). However, despite normal serum levels of a cytokine such as IL-9 pathophysiologic significance may exist. Indeed, the finding that IL-9 has a pathogenic role in psoriasis is coherent with our previous observation in K5.hTGF-β1 transgenic mice, in which the therapeutic response of the psoriasiform skin to PUVA treatment correlated well with the downregulation of IL-9 in the serum [23].

Intradermal injection of IL-9 in WT mice induced local inflammation along with increased expression of IL-17A and STAT3 (Figure 2 and 3). More support for the role of IL-9 in psoriasis comes from our findings in K5.hTGF-β1 transgenic mice. The skin of such mice is marked by hyperplastic epidermis, skin infiltration by neutrophils, T cells and macrophages, basement membrane degradation, increased angiogenesis and multiple cytokine abnormalities similar to those seen in human psoriasis [21–27]. Th17 cells have been implicated in this model, since the pathology can be inhibited by administering 8-methoxypsoralen plus ultraviolet A (PUVA) therapy or by blocking
platelet activating factor, both of which target the Th17 pathway [22,23]. Moreover, we have previously demonstrated that injection with anti-IL-17 antibody was able to stop progression of psoriatic disease in K5.hTGF-β1 transgenic mice [22]. We now found that there was increased IL-9R and IL-9 expression in the skin of K5.hTGF-β1 mice and intradermal IL-9 injection induced Th17-associated skin inflammation, including expression of IL-17A (Figure 1 and 3). In addition, IL-9 may induce IL-17- or IL-22 producing CD4+ T cells in the skin as these cells have been recently reported to play a critical role in IL-23-induced psoriasiform dermatitis in mice [30,31].

IL-9 has been demonstrated to play a role in models of autoimmune disease such as EAE [12,14]. For instance, adoptive transfer of polarized Th9 cells in mice can induce the development of EAE and experimental autoimmune uveitis, through mechanisms distinct from those caused by Th1- and/or Th17-mediated inflammation. Moreover, neutralizing antibodies against IL-9 can delay the development of EAE [12,14]. We found that injecting anti-IL-9 antibody into K5.hTGF-β1 transgenic mice not only diminished the psoriasis-like morphological changes, including cellular infiltration and neo-vascularization of the skin, but also reduced expression of IL-17A (Figure 5). In addition, injecting anti-IL-17 into the K5.hTGF-β1 transgenic mice decreased skin IL-9 mRNA and serum IL-9 protein levels (data not shown). Together, these data suggest a positive feedback loop between IL-9 and IL-17A.

IL-9 is a cytokine with pleiotropic activities, including activity as a growth factor for mast cells and T cells (e.g., Th17 cells), which can secrete pro-angiogenic factors such as IL-8, IL-17, TNF, HGF, FGF-2, and VEGF [32–43]. Effects on these cells may have contributed to our findings that IL-9 promoted angiogenesis and VEGF and CD31 overexpression in vivo (Figure 4). In this regard, it is of interest that we found an effect of IL-9 and anti-IL-9 treatment on mast cells, given recent evidence that mast cells may play a pathogenic role in psoriasis by augmenting VEGF release and thereby increasing inflammation via functional interactions.
with substance P and IL-33 [43]. In addition, we observed that treating K5.hTGF-β1 mice with anti-IL-9 inhibited blood vessel formation (Figure S2) and VEGF expression in skin and soft tissue (Figure 5). This effect was consistent with findings from a mouse study in which anti-IL-9 treatment decreased allergen-induced lung inflammation by reducing VEGF and FGF-2 expression and mast cell numbers in situ [34]. However, we also provided evidence here that IL-9 has direct effects on endothelial cells to induce the formation of new vessels, since IL-9 strongly promoted tube formation by HDMEC in vitro (Figure 4D,E).

Our recent demonstration [24] that non-viral anti-angiogenic gene therapy alleviated the psoriasis-like phenotype in K5.hTGF-β1 mice [at least by part through downregulation of CD31 expression] indicates that the pro-angiogenic activity of IL-9 may be an important component in the role of this cytokine in psoriasis. The potential importance of angiogenesis as a target of anti-psoriatic treatment is consistent with recent reports, indicating that anti-VEGF treatment with monoclonal antibodies such as bevacizumab can lead to remission of psoriasis [44,45].

Taken together, our data suggest that IL-9 has a role in the development of psoriatic lesions through Th17-associated inflammation and angiogenesis. Our data using the K5.hTGF-β1 mice also suggest that psoriasis-like inflammation can be ameliorated by anti-IL-9 treatment. This raises the possibility that similar targeting cytokines such as TNF-α [46], IL-12/23 [47,48], IL-17 [49–51], or IL-21 [52] blocking of IL-9 might be of potential benefit in patients with psoriasis and other Th17 cell-mediated autoimmune diseases.

Supporting Information

Figure S1 Schematic representation of the genes present around IL-9 within the 5q31.1 region. The region is lying within psoriasis susceptibility 11. Search made by NCBI online Mendelian Inheritance in Man (OMIM).

Figure S2 Anti IL-9 treatment reduces inflammatory cell infiltration of the skin and angiogenesis in K5.hTGF-β1 transgenic mice. K5.hTGF-β1 transgenic mice were injected i.p. with either anti-IL-9 antibody or IgG isotype control antibody (n = 5 mice per group) (10 mg/kg) twice a week for 4 weeks and skin samples were collected at the end of week 4 for analysis. WT mice served as controls. (A) Immunohistochemical staining of CD3+ T cells and CD68+ monocytes/macrophages and Giemsa staining of mast cells. (B, C) The skin and adjacent soft tissue of the trunk was prepared for taking photographs from the reverse site in order evaluate the presence of blood vessels. (B) Example shown is from IgG isotype control antibody-injected K5.hTGF-β1 transgenic.
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Author Contributions
Interpreted the data: TPS. Generated the figures: TPS. Originally generated: XJW. Provided the transgenic animals: MPS KW. Discussed the results: TPS MPS KW AGW XJW PW. Read and commented on the manuscript: TPS MPS KW AGW XJW PW. Conceived and designed the experiments: TPS PW. Performed the experiments: TPS. Analyzed the data: TPS. Contributed reagents/materials/analysis tools: PW AGW MPS KW XJW. Wrote the paper: TPS PW.

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