Antagonization of IL-17A Attenuates Skin Inflammation and Vascular Dysfunction in Mouse Models of Psoriasis

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Besides skin inflammation, patients with severe psoriasis suffer from an increased risk of cardiovascular mortality. IL-17A plays a central role in the development of psoriasis and might connect skin and vascular disease. The aim of this study was to clarify whether anti-IL-17A therapy could also ameliorate the vascular dysfunction associated with severe psoriasis. We analyzed three murine models with varying severities of psoriasis-like skin disease concerning their vascular function and inflammation: (i) K14-IL-17Aind/ind mice with keratinocyte-specific IL-17A overexpression and an early-onset severe psoriasis-like phenotype; (ii) homozygous CD11c-IL-17Aind/ind and heterozygous CD11c-IL-17Aind/+ mice overexpressing IL-17A in CD11c+ cells, leading to a delayed onset of moderate psoriasis-like skin disease; and (iii) the acute model of imiquimod-induced psoriasis-like skin inflammation. Similar to the severity of skin disease, vascular dysfunction correlated with peripheral IL-17A levels and neutrophil infiltration into the aortic vessel wall. Successful anti-IL-17A treatment of psoriatic skin lesions diminished peripheral oxidative stress levels, proinflammatory cytokines, and vascular inflammation. These data highlight the pivotal role of IL-17A linking the development of skin lesions and vascular disease in psoriasis. Anti-IL-17A therapy might thus represent a useful approach to attenuate and prevent vascular disease in psoriasis patients.


INTRODUCTION

Psoriasis is the most common chronic skin disease worldwide, affecting up to 6.5% of the European population (Papp et al., 2012). The IL-23/IL-17A axis forms the major immune pathway in the pathogenesis of psoriasis (Di Cesare et al., 2009; Girolomoni et al., 2017). IL-23 secreted by conventional dendritic cells (Singh et al., 2016; Wohn et al., 2013) activates IL-17A-producing γδ T cells and T helper type 17 cells, driving the development and perpetuation of the psoriatic skin lesions (Cai et al., 2011; Pantelyushin et al., 2012). During psoriatic plaque formation, IL-17A and associated proinflammatory cytokines trigger epidermal hyperplasia, excessive keratinocyte proliferation, dermal thickening, and hyperturgescence, as well as the recruitment and activation of neutrophils, monocytes, dendritic cells, and T cells into the skin (Nakajima et al., 2011; Nicklof, 2007; Wohn et al., 2016).

A severe manifestation of psoriasis often requires immunosuppressive therapy (Nast et al., 2015). Monoclonal antibodies targeting IL-17A (secukinumab [Langley et al., 2014] and ixekizumab [Leonardi et al., 2012]) or its receptor (brodalumab [Lebwohl et al., 2015]) represent novel highly selective and efficient treatment options for patients with severe psoriasis (Kurschus and Moos, 2017; Leonardi et al., 2012; Papp et al., 2012; Waisman, 2012). Psoriasis is more than a skin disease and, in particular, patients suffering from severe psoriasis carry an increased risk for cardiovascular mortality, independent of the traditional cardiovascular risk factors such as smoking or hypercholesteremia (Gelfand et al., 2010; Mehta et al., 2010). IL-17A has already been identified as a key factor in both disease patterns: it is not only important in the pathogenesis of psoriasis (Di Cesare et al., 2009) but also contributes to the development of vascular dysfunction and hypertension (Madhur et al., 2010) and has been suggested to connect...
psoriasis and cardiovascular comorbidity (Golden et al., 2013; Vena et al., 2010b). In the vasculature, IL-17A is an essential cytokine contributing to angiotensin II (AngII)-induced vascular dysfunction and hypertension (Madhur et al., 2010; Saleh et al., 2016). Hence, mice lacking IL-17A display reduced vascular inflammation and attenuated vascular dysfunction in response to AngII treatment (Madhur et al., 2010). Conversely, mice overexpressing IL-17A in keratinocytes (K14-IL-17A<sup>ind/+</sup> mice) exhibit severe psoriasis skin inflammation and vascular dysfunction, in conjunction with infiltration of the vasculature by inflammatory myeloid cells. Nonetheless, anti-inflammatory intervention targeting IL-6 or tumor necrosis factor-α showed only limited efficacy in improving vascular dysfunction in K14-IL-17A<sup>ind/+</sup> mice (Karbach et al., 2014).

Here, we sought to study an alternative anti-inflammatory interventional approach by targeting IL-17A in psoriasis with a particular focus on vascular dysfunction, using three established translational models of psoriasis-like skin disease:

(i) K14-IL-17A<sup>ind/+</sup> mice overexpressing IL-17A in K14<sup>+</sup> keratinocytes and developing an early-onset severe psoriatic skin disease correlated with vascular inflammation and hypertension (Croxford et al., 2014; Karbach et al., 2014),

(ii) homozygous CD11c-IL-17A<sup>ind/+</sup> and heterozygous CD11c-IL-17A<sup>ind/+</sup> mice expressing IL-17A in CD11c<sup>+</sup> cells and displaying moderate to severe psoriasis-like skin lesions with a delayed onset (Wohn et al., 2016); and (iii) besides these chronic models mice with imiquimod-induced psoriatic plaque formation, representing an acute short-term model of psoriasis-like skin involvement (van der Fits et al., 2009).

RESULTS

Anti-IL-17A treatment fails to improve skin and vascular disease in mice with severe early-onset psoriasis-like skin disease

To determine whether anti-IL-17A therapy is effective for attenuating vascular disease in a model of severe psoriasis-
Table 1. Skin and Vascular Involvement in the Different Experimental Models of Psoriasis-like Disease Analyzed in This Study

<table>
<thead>
<tr>
<th>Model of Psoriasis-like Skin Disease</th>
<th>Severity of Skin Involvement</th>
<th>Onset of Skin Phenotype (Incidence)</th>
<th>Skin IL-17A Levels (pg/mg protein)</th>
<th>Systemic IL-17A Levels (pg/ml)</th>
<th>Vascular Dysfunction</th>
<th>Anti-IL-17A Treatment Improves Skin Disease</th>
<th>Vascular Dysfunction and Inflammation</th>
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</thead>
<tbody>
<tr>
<td>K14-IL-17Aind/+</td>
<td>Severe and chronic</td>
<td>3 weeks (100%)</td>
<td>1,814.0 ± 300.5</td>
<td>6,730 ± 515.9</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>CD11c-IL-17Aind/+</td>
<td>Moderate to severe and chronic</td>
<td>9–18 weeks (100%)</td>
<td>582.8 ± 67.0</td>
<td>1,440 ± 200.4</td>
<td>Yes (in older mice), partially (in young mice)</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>CD11c-IL-17Aind/ind</td>
<td>Moderate and chronic</td>
<td>18–26 weeks (30%)</td>
<td>385.7 ± 43.77</td>
<td>320.4 ± 31.4</td>
<td>By trend</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>IMQ-induced (C57BL/6J)</td>
<td>Moderate to severe, acute (short-term)</td>
<td>Induced in adult mice (7 weeks old) over 5–7 consecutive days (100%)</td>
<td>271.3 ± 52.5</td>
<td>3.16 ± 1.42</td>
<td>No (only signs of beginning inflammation)</td>
<td>ND</td>
<td>Yes</td>
</tr>
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</table>

Abbreviation: ND, not determined.

Figure 2. Constitutive expression of IL-17A in CD11c<sup>+</sup> cells leads to psoriasis-like skin lesions and vascular dysfunction in a dose-dependent manner. (a) Isometric tension studies of aortic rings of 10–19-week-old CD11c-IL-17A<sup>ind/+</sup>, CD11c-IL-17A<sup>ind/ind</sup>, and control mice in response to ACh. Two-way analysis of variance test and Bonferroni post hoc test, n = 3–14. (b) ROS/RNS measurement of whole blood (20 minutes PDBu stimulation), repeated measurements of pooled samples. Kruskal-Wallis with Dunn multiple comparisons tests, n = 5–11. (c) Flow-cytometric analysis of aortas. Aorta cell number of Ly6G<sup>+</sup>Ly6C<sup>+</sup> neutrophils per 1 cm of aorta is shown. Cells pre-gated on living, CD45.2<sup>+</sup>, and CD11b<sup>+</sup> cells, n = 3–9. Kruskal-Wallis with Dunn multiple comparisons tests. (d) Systemic (plasma) and (e) local (skin) IL-17A levels in 10–19-week-old IL-17A<sup>ind/+</sup>, CD11c-IL-17A<sup>ind/+</sup>, CD11c-IL-17A<sup>ind/ind</sup>, and K14-IL-17A<sup>ind/+</sup> mice; n = 4–6. Kruskal-Wallis with Dunn multiple comparisons tests. IL-17A<sup>ind/+</sup> control compared with CD11c-IL-17A<sup>ind/+</sup> mice: *P < 0.05, **P < 0.01, ***P < 0.001. IL-17A<sup>ind/ind</sup> control compared with CD11c-IL-17A<sup>ind/+</sup> mice: #P < 0.05, ###P < 0.001. ACh, acetylcholine; M, mol/L; ROS/RNS, reactive oxygen and nitrogen species.
like skin disease, we treated K14-IL-17A ind/+ mice with an IL-17A-neutalizing antibody (20 μg/g body weight) versus isotype control starting between 3 and 5 weeks of age over a period of 6 weeks. Under anti-IL-17A treatment, the severity of skin disease was not significantly lessened, as determined by cumulative Psoriasis Area and Severity Index (PASI) score (Figure 1a, and see Supplementary Figure S1a online for single PASI scores). Skin-invading CD45+ leukocytes including CD11b+ myeloid cells, neutrophils (CD11b+Ly6C+Ly6G+), and T cells were not reduced by anti-IL-17A treatment (Figure 1b, and see Supplementary Figure S1c). Reactive oxygen and nitrogen species (ROS/RNS) levels in the blood were slightly but not significantly reduced in the anti-IL-17A antibody-treated mice (Figure 1c).

Therefore, in a second experimental approach, we applied the anti-IL-17A antibody at a higher concentration (60 μg/g body weight) in an attempt to more effectively antagonize the high peripheral IL-17A levels in the K14-IL-17A ind/+ mice (Croxford et al., 2014; Karbach et al., 2014). Even this high-dose treatment was not able to attenuate skin disease in K14-IL-17A ind/+ mice (Figure 1a, and see Supplementary Figure S1b for single PASI scores.) Accordingly, there was no reduction in skin-infiltrating inflammatory cells (total leukocytes, myeloid cells, neutrophils, or T cells) in anti-IL-17A compared to isotype-treated K14-IL-17A ind/+ mice (Figure 1b, and see Supplementary Figure S1c). Neither did peripheral oxidative stress levels decrease upon anti-IL-17A treatment (Figure 1c). As previously described, vascular dysfunction in K14-IL-17A ind/+ mice was correlated with vascular inflammation and infiltration of Ly6C+Ly6G+ neutrophils into the aortic vessel wall (Karbach et al., 2014). High-dose anti-IL-17A treatment caused a small but statistically nonsignificant improvement of endothelial dysfunction (Figure 1d). In agreement, the number of Ly6C+Ly6G+ neutrophils accumulating in the aortic wall was slightly but not significantly reduced in K14-IL-17A ind/+ mice under anti-IL-17A treatment (Figure 1e). Taken together, the K14-IL-17A ind/+ mouse strain exhibits a harsh, psoriasis-like skin phenotype together with severe vascular dysfunction, against which anti-IL-17A treatment in conventional dosages was not effective.

Mice with gradual development of moderate to severe psoriasis-like skin disease also suffer from vascular dysfunction

CD11c-IL-17A ind/ind and CD11c-IL-17A ind/+ mice express IL-17A in CD11c+ cells, which leads to steadily rising systemic IL-17A levels with increasing age (Wohn et al., 2016). The animals acquire a moderate to severe form of psoriasis-like skin disease with a more delayed onset than in the K14-IL-17A ind/+ mice. The kinetics of skin lesion development is IL-17A-dose dependent. In homozygous CD11c-IL-17A ind/ind mice, lesions appear between 9 and 18 weeks of age with 100% incidence, whereas only about 30% of heterozygous CD11c-IL-17A ind/+ mice acquire lesions, starting at 18 weeks age of (Wohn et al., 2016) (Table 1).

Homozogous CD11c-IL-17A ind/ind and heterozygous CD11c-IL-17A ind/+ mice (10–19 weeks old) both showed vascular dysfunction typically associated with psoriasis (Figure 2a). Moreover, peripheral ROS/RNS levels were significantly elevated in the homozygous CD11c-IL-17A ind/ind mice compared with IL-17A ind/+ controls and slightly but not significantly increased in heterozygous CD11c-IL-17A ind/+ animals (Figure 2b). Neutrophil infiltration into the aortic vessel wall was significantly augmented in homozygous CD11c-IL-17A ind/ind and by trend also in heterozygous CD11c-IL-17A ind/+ mice (Figure 2c). In parallel, systemic IL-17A levels were significantly amplified in CD11c-IL-17A ind/ind mice (1,440 ± 200 pg/ml) and by trend also in CD11c-IL-17A ind/+ mice (320.4 ± 31.4 pg/ml) compared with IL-17A ind/+ control animals (10.9 ± 1.03 pg/ml) (Figure 2d). Even in homozygous CD11c-IL-17A ind/ind mice, IL-17A cytokine levels were 4.7-fold lower than in K14-IL-17A ind/+ mice (6,730 ± 1,151 pg/ml). Systemic IL-17A levels showed a parallel increase to local IL-17A levels in the skin (CD11c-IL-17A ind/+ = 385.7 ± 43.77 pg/mg protein, CD11c-IL-17A ind/ind = 582.8 ± 67.0 pg/mg protein, K14-IL-17A ind/ind = 1,814.0 ± 300.5 pg/mg protein) (Figure 2e). Taken together, these data indicate that the severity of both the psoriatic skin lesions and the associated vascular dysfunction represent an IL-17A dose-dependent effect.

Successful anti-IL-17A therapy of late-onset moderate to severe psoriasis-like skin lesions and vascular dysfunction

CD11c-IL-17A ind/ind mice were treated over 4 weeks starting at an age of 12 weeks (i.e., after the appearance of psoriatic skin lesions), with 60 μg/g body weight of anti-IL-17A or isotype control antibody. Antagonizing IL-17A in CD11c-IL-17A ind/ind mice eradicated cutaneous lesions and prevented their reappearance for the duration of the treatment (Figure 3a). Microabscess formation and epidermal thickening combined with hyperkeratosis were absent in CD11c-IL-17A ind/ind mice after 4 weeks of anti-IL-17A injection (Figure 3b). Invasion of neutrophils, a hallmark of psoriatic skin inflammation, and CD11b+ myeloid cells into the ear skin were, respectively, brought back to baseline and attenuated in anti-IL-17A-treated CD11c-IL-17A ind/ind mice (Figure 3c and d). Moreover, vascular function in CD11c-IL-17A ind/ind mice was completely restored by the anti-IL-17A treatment (Figure 3e), and there was a decrease in oxidative stress formation in peripheral blood of anti-IL-17A- but not isotype-treated CD11c-IL-17A ind/ind mice (Figure 3f). Consequently, fewer neutrophils infiltrated the aortic vessel wall of CD11c-IL-17A ind/ind mice under anti-IL-17A treatment (Figure 3g). In peripheral blood, there was a tendency towards reduced levels of the proinflammatory cytokines IL-6, tumor necrosis factor-α (TNF-α), and IL-22, whereas IL-1β levels were not lower after anti-IL-17A antibody treatment (see Supplementary Figure S2a online).

Beyond this remarkable efficacy in a therapeutic setting, applying the anti-IL-17A antibody to younger CD11c-IL-17A ind/ind mice starting at the age of 8 weeks (i.e., before the onset of the skin phenotype), completely prevented skin lesion development (Figure 3h). Epidermal thickness was only slightly increased in younger CD11c-IL-17A ind/ind animals and was brought back to baseline under anti-IL-17A antibody treatment (see Supplementary Figure S3a). Similarly, the elevated frequencies of CD11b+ myeloid cells, Ly6C+Ly6G+ neutrophils, Ly6C+ monocytes, and F4/80+ macrophages all were normalized (see Supplementary Figure S3a).
Figure 3. Anti-IL-17A treatment of CD11c-IL-17Aind/ind mice cures skin and vascular disease in older mice and prevents the development of disease in young animals. IL-17Aind/+ control mice and CD11c-IL-17Aind/ind mice were treated for 4 weeks (60 μg/g body weight anti-IL-17A antibody [BZN035] or isotype control antibody [YB-91-QE90] starting at the age of either (a–e) 12 weeks or (h–j) 8 weeks (young CD11c-IL-17Aind/ind mice without skin lesions). (a) Weekly cumulative PASI score of treated CD11c-IL-17Aind/ind or control mice. Two-way analysis of variance and Bonferroni post hoc test (antibody-treated vs. isotype-
Limited vascular inflammation without vascular dysfunction in murine acute, short-term, psoriasis-like skin disease

To evaluate murine psoriasis-like skin disease independent of genetic IL-17A overproduction, we selected the acute model of imiquimod (IMQ)-induced psoriasis-like skin disease triggered via toll-like receptor 7/8 activation of dendritic cells (Wohn et al., 2013), which is largely dependent on the IL-23/IL-17A axis (van der Fits et al., 2009). Topical application of IMQ over 5 or 7 consecutive days led to the occurrence of erythema, scaling, and thickening of the skin, as previously described (El Malki et al., 2013; van der Fits et al., 2009; Wohn et al., 2013) (Figure 4a and b, and see Supplementary Figure S4 online). Simultaneous injection of anti-IL-17A significantly delayed the onset and attenuated the severity of IMQ-induced skin disease, confirming the relevance of IL-17A in this model (Figure 4a and b, and see Supplementary Figure S4).

While systemic IL-17A levels in the serum were not elevated upon IMQ treatment (Figure 4c), local IL-17A in the skin was significantly increased (Figure 4d), although to a lesser extent than in the different genetic models (Table 1). Accordingly, IMQ-induced infiltration of CD45⁺ leukocytes, including CD11b⁺ myeloid cells, Ly6C⁺Ly6G⁻ neutrophils, Ly6C⁻ monocytes, and F4/80⁺ macrophages into the ear skin was impaired by anti-IL-17A treatment (Figure 4e). Oxidative stress levels in the peripheral blood were increased in mice with IMQ-induced skin disease and slightly but not significantly curtailed by anti-IL-17A (Figure 4f). Proinflammatory IL-6, IL-22, and IL-1β cytokine levels were elevated by trend in IMQ-treated mice and attenuated under anti-IL-17A treatment (see Supplementary Figure S2b). In contrast to the chronic models, vascular function as analyzed by acetylcholine (ACh)-triggered aortic relaxation was not impaired in short-term IMQ-treated mice (data not shown). There was a small increase in inflammatory CD45⁺ leukocytes and CD11b⁺ myeloid cells infiltrating the aortic vessel wall under IMQ treatment, which was partially resolved by anti-IL-17A, and—in line with the unaltered vascular function—the number of Ly6C⁺Ly6G⁺ neutrophils in the aorta was not affected (Figure 4g).

In summary, short-term skin treatment with IMQ raised oxidative stress levels and CD45⁺ inflammatory cells in the aortic vessel wall without mediating vascular dysfunction. The ability of anti-IL-17A to attenuate acute psoriatic skin disease, peripheral oxidative stress levels, and beginning vascular inflammation further supports its feasibility as a prophylactic treatment option.

DISCUSSION

In this study we demonstrate that vascular inflammation and dysfunction in three established models of psoriasis-like skin disease are correlated with the severity of skin lesion manifestation and cutaneous as well as peripheral IL-17A levels (Table 1). The K14-IL-17Aind/ind mice acquire the highest local and systemic IL-17A levels and exhibit a particularly severe psoriasis-like skin phenotype. In addition, they show highly elevated peripheral oxidative stress levels and suffer from vascular dysfunction based on aortic neutrophil infiltration. Homozygous CD11c-IL-17Aind/ind mice and heterozygous CD11c-IL-17Aind/+ mice show a delayed onset of moderate to severe psoriasis-like skin disease associated with reduced amounts of cutaneous IL-17A. In agreement with elevated skin and a stepwise increase in systemic IL-17A (Wohn et al., 2016), homozgyzous CD11c-IL-17Aind/ind mice develop earlier and more severe skin lesions, as well as more pronounced vascular inflammation and dysfunction, than heterozygous CD11c-IL-17Aind/+ mice. These findings are in line with the correlation between the severity of skin disease and systemic IL-17A levels in psoriasis patients (Arican et al., 2005). Moreover, our data establish a direct link between systemic IL-17A levels and the severity of skin disease and vascular inflammation/dysfunction. In fact, the observation that 16-week-old but not in 12-week-old CD11c-IL-17Aind/ind mice exhibit vascular dysfunction suggests that, similar to the formation of skin lesions (Wohn et al., 2016), a certain threshold of systemic IL-17A must be exceeded for vascular dysfunction to develop. Consistent with the central role of IL-17A in the development of vascular dysfunction and inflammation (Madhur et al., 2010; Saleh et al., 2016), our results link the severity of skin disease and the amount of cutaneous and systemic IL-17A with peripheral oxidative stress levels and vascular dysfunction. On the other hand, in the IMQ model of acute moderate to severe psoriatic skin lesion development, we detect only beginning...
Figure 4. Anti-IL-17A treatment in IMQ-induced psoriasis-like skin disease. C57BL/6J mice were treated 5 days with Aldara (MEDA Pharma, Solna, Sweden) cream (5% IMQ) and injected with 0.6 mg anti-IL-17A (BZN035)/isotype control (YB-91-QE90) on days 0 and 3. (a) Single (erythema, scaling, skin thickness) and cumulative PASI scores of IMQ- and sham-treated mice and control mice; n = 13–15, two-way analysis of variance. (b) Hematoxylin and eosin staining of skin sections of IMQ- and sham-treated mice and control mice. One-way analysis of variance and Bonferroni post hoc test. Representative images of n = 10 mice. Scale bar = 100 μm. (c) Plasma and (d) skin IL-17A levels of IMQ-treated mice and sham-treated control mice; n = 10, Mann-Whitney t test in c; n = 5,
vascular inflammation, together with elevated peripheral oxidative stress levels. This is in accordance with the short-term nature of psoriatic skin disease, which leads to only moderately up-regulated IL-17A levels in the skin and peripheral blood (Table 1). Thus, it may be useful to study vascular inflammation/dysfunction in a prolonged IMQ-induced disease model (Terhorst et al., 2015). Aortic neutrophil infiltration in the presented genetic psoriatic skin disease models is lower than in mice with AngII-induced vascular dysfunction (Wenzel et al., 2011); nevertheless, they exhibit comparable vascular dysfunction. The aortic inflammation in the genetic IL-17Aind/ind psoriasis mouse models occurs over a longer period of time than in mice treated with AngII. It is tempting to speculate that additional effects of IL-17A other than neutrophil infiltration may contribute to impaired vascular function.

Our results are consistent with reports of human studies indicating that patients with severe psoriasis have an increased risk of cardiovascular mortality that is independent of the traditional cardiovascular risk factors (Mehta et al., 2010; Vena et al., 2010a; Vena et al., 2010b). Specifically, severe psoriasis confers an additional 6.2% absolute risk of a 10-year rate of major adverse cardiac events compared with the general population (Mehta et al., 2011a). The mechanisms of this association remain elusive, as does the possible impact that current treatment protocols of psoriasis may have on the cardiovascular risk and disease. Likewise, the role of systemic immunotherapy on cardiovascular disease development in autoimmune disease patients remains to be elucidated in further studies (Takata et al., 2011; Zhang et al., 2016). Recently, evidence for beneficial effects of anti-inflammatory treatment with a monoclonal antibody targeting IL-1β in patients with known coronary artery disease reinforced the importance of inflammation and possible anti-inflammatory antibody treatment in cardiovascular disease (Ridker et al., 2017a, 2017b). The cytokine IL-17A, which can be directly antagonized for effective treatment of psoriasis, forms one possible connection between skin and vessel disease (Golden et al., 2013; Vena et al., 2010b). IL-17A does not only promote neutrophil infiltration into the skin (Di Cesare et al., 2009) but also into the aortic vessel wall (Karbach et al., 2014) and thereby leads to vascular dysfunction and hypertension (Madhur et al., 2010). In line with this, psoriasis patients exhibit increased vascular inflammation in multiple segments of the aorta compared with healthy controls (Mehta et al., 2011b).

In this study, anti-IL-17A treatment was not effective in the very severe K14-IL-17Aind+/psoriatic skin disease model to ameliorate skin nor vascular disease. This may be due to the very early (data not shown) and particularly high peripheral IL-17A levels. In CD11c-IL-17Aind/ind mice that produce about 4.7-fold lower amounts of IL-17A, anti-IL-17A was highly effective for treating cutaneous and associated vascular inflammation/dysfunction. Both skin and vascular disease were, respectively, completely cured and prevented when anti-IL-17A was applied therapeutically and prophylactically. These findings may have vital therapeutic implications for cardiovascular risk prevention and management in psoriasis patients. In fact, anti-IL-17A therapy may not only lower the risk of cardiovascular complications in patients with severe psoriasis as is currently done, but it may also be warranted to attenuate vascular inflammation and prevent cardiovascular disease when applied earlier, that is, in moderate psoriasis. Further supporting this therapeutic strategy, antibodies to IL-17A or the IL-17 receptor A subunit lowered blood pressure by 30 mm Hg and ameliorated vascular inflammation in a model of AngII-induced arterial hypertension (Saleh et al., 2016). Moreover, Li et al. (2018) recently described that arterial thrombus formation was attenuated in a mouse model of psoriasis under IL-17A inhibition, indicating that targeting cytokines that mediate psoriatic inflammation may indeed improve cardiovascular comorbidities. Our data demonstrate that psoriasis-associated vascular inflammation/dysfunction can potentially be cured and prevented by anti-IL-17A treatment.

From a clinical perspective, our study underlines the need to keep vascular disease and dysfunction in mind when treating psoriasis patients (Gelfand et al., 2011; Mehta et al., 2010). In different psoriasis-like mouse models, we could establish a direct link between the severity of skin involvement, local and circulating IL-17A cytokine levels, and vascular inflammation/dysfunction. Hence, our findings suggest that in psoriasis, the vasculature reacts in a similar way as the skin. IL-17A–mediated growing skin inflammation and plaque formation correlate with increasing inflammation of the vessel wall, leading to impaired vascular function, and most likely the development of hypertension and, eventually, increased cardiovascular mortality. Thus, psoriasis patients need to be educated properly about their increased risk of cardiovascular disease. Moreover, a combined dermatological-cardiological treatment may be warranted, including regular blood pressure controls and adequate therapy in the case of hypertension, to limit this life-limiting comorbidity of psoriasis (No et al., 2017).

In conclusion, our mouse data indicate that anti-IL-17A treatment of psoriasis may have a similar beneficial effect on the coexisting vascular inflammation. In addition, our psoriasis mouse models based on transgenic overexpression of IL-17A represent valuable tools for investigating the effects of biological therapies on cardiovascular disease in psoriasis. Our findings warrant future long-term studies in psoriasis patients to better understand the effects of biologicals, in particular IL-17A therapies, on cardiovascular comorbidity.

MATERIALS AND METHODS

Mouse models of psoriasis-like skin disease

IL-17Aind/ind mice were either crossed to K14-Cre or CD11c-Cre to obtain K14-IL-17Aind+/ (Croxford et al., 2014), CD11c-IL-17Aind/+, and unpaired Student t test in d, e. Flow cytometric analysis of ear skin. One-way analysis of variance and Bonferroni post hoc test; n = 14–15. f. ROS/RNS measurement in whole blood (20 minutes of stimulation with PDBu), repeated measurements of pooled samples. One-way analysis of variance and Bonferroni post hoc test; n = 8–9. g. Flow cytometric analysis of aortas of IMQ-treated mice plus anti-IL-17A/isotype- and sham-treated control mice. Kruskal-Wallis and Dunn multiple comparison tests, n = 15. *P < 0.05, **P < 0.01, ***P < 0.001. IMQ, imiquimod; PASI, Psoriasis Area and Severity Index; ROS/RNS, reactive oxygen and nitrogen species.
Psoriasis Area and Severity Index

The severity of psoriasis-like skin disease was determined by modified PASI scoring, as described previously (El Malki et al., 2013; Karbach et al., 2014). Briefly, erythema and scaling were scored (score range = 0–4), skin thickness of the ears and the back skin were measured using a caliper (μm), and the percentage of affected skin was determined. The cumulative PASI score was calculated for the K14-IL-17Aind/+ and CD11c-IL-17Aind/ind mice as follows: (erythema score + scaling score + skin thickness change [%]) × affected area [%]. For IMQ experiments, the percentage of affected skin is not included in the scoring because it depends on the treated area. Hence, the modified PASI is calculated as erythema score + scaling score + skin thickness change (%). In the figures, the cumulative PASI score and/or the single scores for skin thickness, scaling, and erythema are depicted.

Anti-IL-17A treatment

We received the anti-IL-17A antibody from Novartis (clone, BZN035; isotype control, YB-91-QE90) and used the following treatment regimens: Both K14-IL-17Aind/+ and CD11c-IL-17Aind/ind mice were injected twice a week with a dosage of 20 μg/g body weight or 60 μg/g body weight of anti-IL-17A over a period of 4–6 weeks. In the acute IMQ-induced psoriasis-like skin disease model, 0.6 mg anti-IL-17A/induction was applied twice (days 0 and 3) during the 5-day and three times (days 0, 3, and 6) during the 7-day IMQ treatment protocols, respectively. Anti-IL-17A treatment did not affect the weight of the mice (data not shown).

Detection of ROS/RNS formation with L012-enhanced chemiluminescence

Oxidative burst of white blood cells in the whole blood was determined by 8-amino-5-chloro-7-phenylpyrido[3,4-d]pyridazine-1,4-(2H,3H)dione sodium salt (L012) -enhanced chemiluminescence. After injection of 200 IU of heparin into the beating heart of the anesthetized mouse, venous blood was drawn from the right ventricle. Enhanced chemiluminescence was counted in a volume of anesthetized mouse, venous blood was drawn from the right ventricle. Enhanced chemiluminescence was counted in a volume of

Cytokine detection

Plasma cytokine concentrations of IL-17A, IL-6, IL-22, tumor necrosis factor-α, and IL-1β were determined by Luminox Multiplex Assay according to the manufacturer’s instructions (Thermo Fisher Scientific). For skin IL-17A detection, back skin of mice was isolated and triturated with a homogenizer (Kinematica, Bohemia, NY). Cell debris was precipitated via centrifugation (10,000 g, 4 °C, 10 minutes), and IL-17A was measured in the supernatant using the IL-17A DuoSet ELISA kit (R&D Systems) and normalized to the total protein concentration, which was determined via Bradford assay.

Statistical analysis

Statistical analysis was performed with GraphPad Prism software, version 7 (GraphPad Software, La Jolla, CA). First, the data were analyzed for normal distribution (Kolmogorov-Smirnov test). When normal distribution was given, we applied the one-way analysis of variance test with Bonferroni post hoc test. If no normal distribution was given, Kruskal-Wallis test with Dunn multiple comparison or comparison of selected columns was used as appropriate and indicated in the figure legends. PASI scores and aortic relaxation curves were analyzed by two-way analysis of variance with Bonferroni post hoc test. P values of less than 0.001, 0.01, and 0.05 were considered statistically significant. Data are presented as mean ± standard error of the mean.

ORCID

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CONFLICT OF INTEREST
FK is an employee of Novartis. AW is a consultant to Novartis. The other authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2018.09.021.

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