Interleukin 17 Drives Vascular Inflammation, Endothelial Dysfunction, and Arterial Hypertension in Psoriasis-Like Skin Disease

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Objective—Interleukin (IL)-17A is regarded as an important cytokine to drive psoriasis, an inflammatory skin disease marked by increased cardiovascular mortality. We aimed to test the hypothesis that overproduction of IL-17A in the skin leading to dermal inflammation may systematically cause vascular dysfunction in psoriasis-like skin disease.

Approach and Results—Conditional overexpression of IL-17A in keratinocytes caused severe psoriasis-like skin inflammation in mice (K14-IL-17A ind/+ mice), associated with increased reactive oxygen species formation and circulating CD11b+ inflammatory leukocytes in blood, with endothelial dysfunction, increased systolic blood pressure, left ventricular hypertrophy, and reduced survival compared with controls. In K14-IL-17A ind/+ mice, immunohistochemistry and flow cytometry revealed increased vascular production of the nitric oxide/superoxide reaction product peroxynitrite and infiltration of the vasculature with myeloperoxidase+CD11b+GR1−F4/80− cells accompanied by increased expression of the inducible nitric oxide synthase and the nicotinamide dinucleotide phosphate (NADPH) oxidase, nox2. Neutrophil depletion by anti-GR-1 antibody injections reduced oxidative stress in blood and vessels. Neutralization of tumor necrosis factor-α and IL-6 (both downstream of IL-17A) reduced skin lesions, attenuated oxidative stress in heart and blood, and partially improved endothelial dysfunction in K14-IL-17A ind/+ mice.

Conclusions—Dermal overexpression of IL-17A induces systemic endothelial dysfunction, vascular oxidative stress, arterial hypertension, and increases mortality mainly driven by myeloperoxidase+CD11b+GR1−F4/80− inflammatory cells. Depletion of the GR-1+ immune cells or neutralization of IL-17A downstream cytokines by biologicals attenuates the vascular phenotype in K14-IL-17A ind/+ mice. (Arterioscler Thromb Vasc Biol. 2014;34:2658-2668.)

Key Words: animal model of human disease • immune system • inflammation • vascular dysfunction

Psoriasis is the most common chronic inflammatory skin disease affecting up to 6.5% of the population. An important comorbidity of patients with psoriasis is cardiovascular disease (CVD), including coronary heart disease, stroke, peripheral artery disease, and heart failure. Severe psoriasis is meanwhile considered an independent risk factor for cardiovascular mortality, contrasting older data describing CVD risk in psoriasis patients to be primarily a consequence of a higher prevalence of established cardiovascular risk factors, like obesity, smoking, and depression. Interleukin (IL)-17A, a member of the IL-17 family of cytokines, plays a role in the development of vascular dysfunction, hypertension, and pathogenesis of psoriasis. It was found in skin lesions of psoriatic patients and of chemically...
induced models for psoriasis in mice.\textsuperscript{14,16–18} IL-17A was formerly thought to be only generated by a subset of CD4\(^+\) T cells (Th17), but by now we know that also dendritic cells, natural killer cells, macrophages, and γδ-T cells are able to generate IL-17A.\textsuperscript{22} Members of the IL-17 family were described to be produced also by keratinocytes, endothelial cells, and neurons.\textsuperscript{9}

The efficacy of IL-17A neutralization and anti-IL-17-receptor antibody therapy in human psoriasis\textsuperscript{2,20} and the finding that mice lacking the IL-17 receptor alpha develop blunted imiquimod-induced psoriasis-like skin disease as compared with control mice\textsuperscript{21} suggest that IL-17A is an important pro-inflammatory cytokine in the pathogenesis of psoriasis. To further investigate these mechanisms, we generated a mouse strain conditionally overexpressing IL-17A in keratinocytes (K14-IL-17A\textsuperscript{ind/+} mice).\textsuperscript{22} In a conditional knock-in approach, we introduced the targeting construct (IL-17A cDNA) into the chicken β-actin promoter.\textsuperscript{23} The resulting K14-IL-17A\textsuperscript{ind/+} mice, has a skin phenotype mimicking many hallmark features of human psoriasis (Figure ID in the online-only Data Supplement). There is growing evidence that inflammation in the skin may also affect the vasculature\textsuperscript{24} and that IL-17A could be of importance in linking skin disease to cardiovascular disease/inflammation.\textsuperscript{8} As severe psoriasis (but not mild psoriasis) is considered to be an independent risk factor for cardiovascular mortality,\textsuperscript{8} the K14-IL-17A\textsuperscript{ind/+} mice which develop a severe form of psoriasis-like skin disease seemed to be an ideal model to study the correlation between psoriasis and vascular disease in the mouse model. We therefore sought to determine whether IL-17A drives vascular inflammation and dysfunction in severe psoriasis-like skin inflammation using the K14-IL-17A\textsuperscript{ind/+} mice.

We here show that overexpression of IL-17A in keratinocytes induces systemic vascular inflammation, endothelial dysfunction, and arterial hypertension driven by MPO\(^+\) cells, leading to increased mortality. We also confirmed in a cohort of patients with severe psoriasis an independent correlation of psoriasis with arterial hypertension. Short-time depletion of neutrophil granulocytes by anti-GR-1 antibody injection and long-term pharmacological antagonization of tumor necrosis factor-α (TNF-α) and IL-6 attenuated oxidative stress and partially vascular disease in K14-IL-17A\textsuperscript{ind/+} mice. The sequence of events downstream of IL-17A could be mechanistic players to target cardiovascular sequelae of psoriasis.

**Material and Methods**

A description of the material and methods is provided in the online-only Data Supplement.

**Results**

**Impaired Vascular Function and Increased Reactive Oxygen Species Formation in Mice With Psoriasis-Like Phenotype**

In mice, Cre-mediated IL-17A overexpression in keratinocytes results in skin inflammation\textsuperscript{22} (Figure IA in the online-only Data Supplement) comparable to severe human psoriasis displaying hallmark features of the disease like an acanthotically thickened epidermis, hyper- and parakeratosis, multiple (epi)dermal neutrophilic abscesses, increased vessel formation in the skin, and an accumulation of CD11b\(^+\), F4/80\(^+\), and GR1\(^+\) inflammatory myeloid cells in the inflamed skin (Figure IB, IC, and ID in the online-only Data Supplement). K14IL-17A\textsuperscript{ind/+} mice also present with typical comorbidities of psoriasis-like conjunctivitis and arthritis (Figure ID in the online-only Data Supplement) stressing the realistic patho-physiological relevance of this mouse model. Any important ectopic expression of IL-17A (other than keratinocyte-derived) was ruled out using in vivo imaging technology with reporter-Cre strains (see Figure II in the online-only Data Supplement). Thus, the K14IL-17A\textsuperscript{ind/+} mice represent a useful instrument for the experimental analysis of psoriasis-like skin disease and its sequelae.

Vascular relaxation studies of the aortas revealed severe endothelial dysfunction as demonstrated by the decreased responsiveness to the endothelium-dependent vasodilator acetylcholine in K14IL-17A\textsuperscript{ind/+} mice (Figure 1A). Compared with controls, reactive oxygen species (ROS) levels were higher in the blood of K14IL-17A\textsuperscript{ind/+} mice at basal level and on stimulation with the phorbol ester phorbol 12,13-dibutyrate, a protein kinase C–dependent activator of the superoxide producing enzyme nicotinamide dinucleotide phosphate (NADPH) oxidase (Figure 1B). We also found increased NADPH oxidase activity in cardiac homogenates of the K14IL-17A\textsuperscript{ind/+} mice compared with control mice (Figure 1C). Elevated blood ROS levels were accompanied by increased number of circulating CD11b\(^+\)—mainly GR-1\(^+\)—myelomonocytic cells (Figure 2A and 2B) because of activation via their NADPH oxidase.\textsuperscript{25} Systemically, the numbers of both Ly6G\(^+/\)CD11b\(^+\) and Ly6C\(^+/\) CD11b\(^+\) leukocytes were significantly increased (Figure 2C and 2D).

In parallel, we established increased IL-17A levels in the serum of the K14IL-17A\textsuperscript{ind/+} mice (Figure ID in the online-only Data Supplement). Also for humans, it has been reported that serum IL-17 is higher in psoriasis patients compared with healthy controls.\textsuperscript{26} A positive correlation between the psoriasis area and severity index score and the serum IL-17 level in psoriasis vulgaris was described.\textsuperscript{26} IL-6 serum levels were increased in the K14IL-17A\textsuperscript{ind/+} mice (Figure ID in the online-only Data Supplement), and TNF-α was elevated by trend in the serum and in the skin (Figure IIIA and IIIB in the online-only Data Supplement). These cytokines have been described to be elevated...
in the serum of humans with psoriasis in comparison to healthy controls too. Besides their psoriasis-like skin disease, the K14IL-17Aind/+ mice provide a phenotype of vascular dysfunction and elevated oxidative stress levels within the vasculature.

Increased Vascular Oxidative Stress and Reduced Vascular Nitric Oxide Bioavailability in K14IL-17Aind/+ Mice

Endothelial dysfunction in the setting of the psoriasis-like skin disease was accompanied by increased vascular superoxide \( (O_2^-) \) levels throughout the aorta (Figure 3A) and by higher levels of 3-nitrotyrosine in intima and the media (Figure 3B) compatible with increased formation of the nitric oxide (NO-)O_2^- reaction product peroxynitrite (ONOO^-). Inducible NO synthase (iNOS) was found to be upregulated in the outer part of the aortic vessel wall of K14IL-17Aind/+ mice compared with control mice (Figure 3C). We also found elevated levels of serum nitrite (Figure IIC in the online-only Data Supplement). Increased peroxynitrite formation causes, for example, tyrosine nitration of the prostacyclin synthase, uncoupling of the NO-synthase, and inhibition of the soluble guanylyl cyclase, contributing to vascular endothelial dysfunction. Decreased vascular NO bioavailability leads to an inhibition of the NO/cGMP/cGK-I signaling pathway as indicated by a reduced vasodilator-stimulated phosphoprotein phosphorylation in aortas from K14IL-17Aind/+ mice (Figure 3D). Increased oxidative stress within the vasculature was accompanied by an increased expression of the oxidative stress response protein hemeoxygenase-1 in aortas (Figure 3E).

Infiltration of Neutrophils to the Skin Is Accompanied by Increased Levels of Neutrophil Granulocytes in the Aorta of K14IL-17Aind/+ Mice

Besides an upregulation of the catalytic subunit of the phagocytic NADPH-oxidase (gp91phox) in aortas of K14IL-17Aind/+ mice (Figure 4A), myeloperoxidase (MPO)-positive vesicles or cellular structures were increased in the adventitial layer neighboring the perivascular adipose tissue (Figure 4B). CD11b+GR1+F4/80+ cells—most likely representing neutrophil granulocytes—accumulated in the aortas of the K14IL-17Aind/+ mice (Figure 4C). These cells were also upregulated in the skin (Figure IB in the online-only Data Supplement) reminiscent of the accumulation of neutrophil granulocytes seen in the skin lesions of psoriasis patients.

IL-17A-Driven Psoriasis-Like Disease Is Accompanied by Arterial Hypertension, Cardiomyocyte Hypertrophy, and Premature Death

Vascular dysfunction in the K14IL-17Aind/+ mice was accompanied by an increased systolic blood pressure compared with controls (Figure 5A). Renal function was not altered (Figure IVA in the online-only Data Supplement). Most likely as a consequence of arterial hypertension, we detected a significant increase in the heart weight of the K14IL-17Aind/+ mice compared with controls, resulting in a significantly elevated heart-body ratio (Figure 5B). There was no evidence for myocarditis in K14IL-17Aind/+ mice compared with controls (upper panels); only perivascular inflammation was visible in K14IL-17Aind/+ mice (lower panel; Figure 5C), as well as a significant cardiomyocyte hypertrophy (Figure 5C and 5D) in K14IL-17Aind/+ mice. Compared with healthy littermate control animals, K14IL-17Aind/+ mice showed a reduced lifespan (Figure 5E). The mice seemed to die from sudden causes because death occurred without previous prefinal morbidity or decay of the mice. Neither histological analysis of the different organs of the K14IL-17Aind/+ mice nor the analysis of the clinical chemistry laboratory values of the blood of the K14IL-17Aind/+ mice delivered clear evidence for the cause of the earlier death (Figure V in the online-only Data Supplement), making sudden cardiac death a possible reason for the increased mortality of K14IL-17Aind/+ mice.

Increased Cardiovascular Risk Profile in Psoriasis Patients

In a translational approach, we calculated the 10-year risk for development of CVD (as indexed by coronary heart disease,
stroke, peripheral artery disease, or heart failure) by a score based on the Framingham Heart Study. Retrospectively, we found an elevated risk for the hospitalized psoriatic patients of the Department of Dermatology, University Medical Center Mainz, compared with an age- and sex-matched population-based sample without psoriasis from the Gutenberg Health Study (P=0.0062; n=125 psoriasis patients and 375 healthy controls). The relative frequency to belong to the medium or high-risk cardiovascular risk group was higher in the hospitalized psoriasis patients than in the control group (Figure VIA in the online-only Data Supplement). Psoriasis patients were more likely to have hypertension (P=0.0019). Although the

Figure 2. K14-IL-17Aind/+ mice have increased systemic neutrophil granulocytes. A, Blood of K14-IL-17Aind/+ and control mice was analyzed for CD11b, F4/80, and GR1 via flow cytometric analysis, after outgating dead cells and pregating on the B220− and CD3−/CD90.2− cells. One representative plot is shown per group. Percentage given in the plot itself is related to the pregate. (IL-17Aind/+ versus K14-IL-17Aind/+: 65.4% ± 6.6% versus 80.0% ± 4.9% CD11b+, 31.9% ± 4.4% versus 82.8% ± 1.0% GR-1+ of CD11b+, 22.1% ± 7% versus 5.3% ± 1.6% F4/80+ of CD11b+; n=10 mice per group). B, Statistics for the individual populations are calculated as percentage of the living cells using the Student t test (IL-17Aind/+ versus K14-IL-17Aind/+: 16.6% ± 1.4% versus 55% ± 3.4% CD11b+ of all life cells in total blood, 4.8% ± 0.5 versus 45.7% ± 3.0% GR-1+ of all life cells in total blood, 3.6% ± 1.4% versus 3.1% ± 1.0% F4/80+ of all life cells in total blood; n=10 mice per group). C, Statistical analysis is given for the percentage of CD11b+ Ly6G+ Ly6C+ and the Ly6C+ Ly6G− cells in the blood of K14-IL-17Aind/+ and control mice analyzed by flow cytometry analysis (Student t test, n=4–5 mice per group). The representative plots are shown in Figure 6A. D, Splenocytes of K14-IL-17Aind/+ and control mice were analyzed for CD11b, Ly6G, and Ly6C. Dead cells were out-gated, and it was pregated on the B220− and CD90.2− cells. One representative plot is shown per group. Percentage given in the plot itself is related to the pregate. Statistical analysis below shows the total cell number of the CD11b+, Ly6G+, and Ly6C− cells per spleen (n=7 mice per group, Student t test).
psoriasis patients more often had other classical cardiovascular risk factors like smoking, diabetes mellitus, or obesity (all \( P < 0.0001 \); Figure VIB in the online-only Data Supplement; n=418 psoriasis patients versus n=1254 healthy controls), hypertension was shown to be independently associated with psoriasis in a conditional logistic regression model adjusted...
for classical cardiovascular risk factors (P≤0.003; Figure VIC in the online-only Data Supplement). The odds ratio for hypertension among psoriasis patients versus the nonpsoriatic sample was 1.6 (95% confidence interval, 1.2–2.0; P<0.001) in the univariable model and 1.7 (95% confidence interval, 1.2–2.4; P=0.003) in the multivariable model (n=418 psoriasis patients and 1254 healthy controls).

Neutrophil Depletion Leads to a Reduction of Oxidative Stress Levels in the Blood

Systemic application of the antigranulocyte receptor-1 monoclonal antibody RB6-8C5 (anti-GR1) over 24 hours lead to a systemic depletion of the Ly6G+CD11b+ neutrophil granulocytes and partially also of the Ly6G−Ly6C+CD11b+ cells in the blood (Figure 6A). ROS levels in the blood of the K14IL-17Aind/+ mice were significantly reduced after anti-GR1 treatment (Figure 6B), suggesting that increased ROS serum levels in the K14IL-17Aind/+ mice are at least partially mediated by neutrophil granulocytes. Neutrophil granulocytes were also reduced in the spleen (data not shown) and exemplarily in 3 pooled aortas under application of anti-GR1 (Figure 6D). In parallel, a reduction of the vascular superoxide levels in the aortas of the K14IL-17Aind/+ mice compared with untreated K14IL-17Aind/+ mice was seen (Figure 6C).

Blockade of Cytokines Downstream of IL-17A Attenuates Both Skin Disease and Vascular Phenotype in K14IL-17Aind/+ Mice

Because anti-TNFα (etanercept) is frequently used to treat patients with severe psoriasis and TNF-α tended to be increased in the serum and the skin of K14IL-17Aind/+ mice (Figure IIIA in the online-only Data Supplement), the K14IL-17Aind/+ mice were treated for a maximum of 10 weeks with anti-TNFα. As dermal and systemic levels of IL-6 were increased in K14IL-17Aind/+ mice and we had already been able to show an improvement of skin pathology with reduced skin thickness and less infiltrating CD11b+ cells under anti-IL6 treatment (Figure ID in the online-only Data Supplement), we also used anti-IL-6 in another approach, which is approved for the treatment of refractory rheumatoid arthritis, for further analysis. Both treatment regimen led to a significant improvement in skin pathology shown by reduced psoriasis area and severity index score (Figure 7A), reduced MPO+ cell infiltration under anti-TNFα treatment (Figure 7B), and reduced skin thickness under anti-IL6. Importantly, in addition to the improvement of the skin pathology, ROS levels in the blood and cardiac NADPH oxidase activity of K14IL-17Aind/+ mice were mildered by etanercept and by anti-IL-6 (Figure 7C and 7D). In response to etanercept treatment, endothelial dysfunction of the K14IL-17Aind/+
Elevated blood pressure, compensatory heart hypertrophy, and reduced life span in K14-IL-17Aind/+ mice.

Figure 5. Elevated blood pressure, compensatory heart hypertrophy, and reduced life span in K14-IL-17Aind/+ mice. **A** Increased systolic blood pressure in K14-IL-17Aind/+ mice versus IL-17Aind/+ controls measured noninvasively by tail-cuff method (n=6 mice; Student t test; ≥3 measurements per mouse). **B** Heart weights and heart-body ratios of IL-17Aind/+ and K14-IL-17Aind/+ (n=20–22 mice per group; Student t test). **C** Hematoxylin and eosin staining of hearts of K14-IL-17Aind/+ mice compared with IL-17Aind/+ mice (n=5–6; Student t test). **D** Cardiomyocytes per sight field were counted (representative pictures and statistical analysis of n=6 mice per group; Student t test). **E** Survival of IL-17Aind/+ and K14-IL-17Aind/+ mice shown in a Kaplan-Meier curve (n=14 mice per group).

mice was attenuated (Figure 7E), and under IL-6 also a slight improvement was noticed. These findings clearly strengthen the concept that both skin and vascular disease are closely linked by inflammatory mechanisms.

**Discussion**

We demonstrate here with an experimental approach a causative link between dermal IL-17A production in the skin and systemic vascular dysfunction. Our findings demonstrate the feasible role of IL-17A in linking skin and vascular disease, cytokine dissemination, and the knock-on effect of neutrophil granulocytes invading the vessel wall to induce vascular oxidative stress, inflammation, and dysfunction, leading to arterial hypertension and even premature death.

The K14IL-17Aind/+ mice have elevated IL-17A levels in the serum—most likely because of the permeability of the skin. This is comparable to psoriasis patients, where a positive correlation between the psoriasis area and severity index score and the serum IL-17 level has been described.26,27 Elevated IL-17A serum levels have also been reported for the imiquimod-induced psoriasis-like skin disease (=20–40 pg/mL in IMQ-treated wildtype mice, barely detectable in control mice),36 although less in comparison to the K14IL-17Aind/+ mice, which can be explained by the smaller area of affected skin. The elevated IL-17A levels in the serum of the K14IL-17Aind/+ mice were accompanied by increased numbers of CD11b+ cells—mostly neutrophil granulocytes—in the blood (Figure 2A–2C). Independently of psoriasis, it has been shown that IL-17 induces hypertension by decreasing endothelial production of nitric oxide and that IL-17–mediated endothelial dysfunction can be normalized by an IL-17 neutralizing antibody.37 Nuygen et al thus claimed that inhibitors of IL-17 may be useful as antihypertensive drugs in IL-17–associated autoimmune diseases.37

Vascular dysfunction and hypertension in the K14-IL-17Aind/+ mice was associated with enhanced vascular O2− and ONOO− production, presumably driven by phagocytic NOX-2 (NADPH-oxidase 2) and a simultaneous upregulation of iNOS expression. We also found increased MPO levels in aortas of K14IL-17Aind/+ mice. MPO, mostly set free by degranulation of leukocytes, is known to have a profound adverse effect on vascular tone and resistance of vessels38 because of its capacity to oxidize NO. In the K14IL-17Aind/+ mice, CD11b+GR1+ F4/80− cells are elevated in the aorta. IL-17A is a crucial cytokine in neutrophil activation and recruitment.9,39 The invading neutrophil granulocytes might be a cellular source of both MPO and NADPH-oxidase in the animal model of K14IL-17Aind/+ mice, causing vascular inflammation and dysfunction. In line with this, a pilot patient study showed evidence for vascular inflammation in the aortas of patients with moderate-to-severe psoriasis compared with matched healthy controls.40 It was shown recently that in angiotensin II–induced arterial hypertension, aortas were infiltrated with CD11b+Gr-1− F4/80− macrophages and CD11b+Gr-1− F4/80− neutrophils and that ablation of lysozyme-M’CD11b+ myelomonocytic cells improved vascular dysfunction and reduced arterial hypertension.41 Especially, neutrophil-derived IL-6 or cathelicidin CRAMP can act as a switch between neutrophil and monocyte recruitment,42 leading to an enhanced rise in proinflammatory macrophages in the inflamed tissue vessel wall. The fact that short-term neutrophil depletion by anti-GR-1 improved vascular oxidative stress, but not vascular function could be because of knock-on effects of neutrophils on other inflammatory cells, which could not be reversed by the brief duration of the treatment. We therefore suggest that hypertension in K14IL-17Aind/+ mice might result from the vascular dysfunction/inflammation, accompanied by myocardial hypertrophy and increased cardiac NADPH oxidase activity (Figure 1C).

Our experiments antagonizing cytokines downstream of IL-17A signaling point to a possible benefit of specific anti-inflammatory treatment to attenuate not only skin disease, but also—at least partially—the related vascular disease. Small clinical trials made similar observations, showing that anti–TNF-α treatment improved vascular dysfunction in rheumatoid arthritis,43 another IL-17A triggered autoimmune disease accompanied by CVD. But effects of biological treatment on the vascular system have to be considered cautiously. Although a cardio-protective effect has been noticed under anti–TNF-α treatment.
Figure 6. Reduction of ROS in the blood by treatment with anti-GR1. A, CD11b⁺ cells as well as CD11b⁺ Ly6G⁺ Ly6C⁺ neutrophil granulocytes and partially CD11b⁺ Ly6C⁻ Ly6G⁻ cells were depleted in K14-IL-17Aind/+ mice treated over 24 hours with the anti-GR-1 antibody RB6-8C5 by once injecting 150 μg of antibody per mouse intraperitoneally. Depletion was controlled by flow cytometry analysis of the following: after pregating on the living cells, out-gating the B220⁺ and CD90.2⁺ cells and then gating on the CD11b⁺ cells, the Ly6G⁺ and Ly6C⁺ subpopulations are shown here. (Representative panels are shown of n=7–8 mice per group. FMO controls are given.) B, Oxidative burst after Zymosan and phorbol 12,13-dibutyrate (PDBU) incubation was measured in the blood of IL-17Aind/+ control mice and K14-IL-17Aind/+ mice with or without anti-GR-1 treatment. (n=16 measurements of 7–8 mice per group, pooled samples, 1-way ANOVA by guest on June 24, 2017 http://atvb.ahajournals.org/ Downloaded from
in psoriasis and methotrexate in psoriasis or rheumatoid arthritis, increased cardiovascular events under biological treatment of psoriasis with antibodies to the shared p40 subunit of IL-12 and interleukin-23 as potential downstream effectors of IL-17 have been described. IL-17A has been characterized as a cytokine that triggers hypertension, vascular dysfunction, and also atherosclerosis. Taleb et al demonstrated that in vivo administration of IL-17 reduces vascular T cell infiltration and limits atherosclerosis development. However, IL-17A can also confer protection to the vasculature in the continuum of atherogenesis and its consequences, such as plaque rupture. Gistera et al could show a decreased stability of atherosclerotic plaques when inhibiting IL-17 through neutralizing antibodies, suggesting that patients treated with IL-17 receptor blockers should be closely monitored for the appearance of cardiovascular events. These findings show that the role of IL-17A in atherosclerosis is multifunctional and reflects the outbalanced immunologic response in this complex chronic inflammatory disease. Therefore, more studies will be needed to understand whether the GR-1+ immune cells or the cytokines downstream of IL-17A might be useful targets to treat the cardiovascular sequela of severe psoriasis.

Our patient survey confirmed the correlation between psoriasis and the prevalence of hypertension, myocardial infarction, and coronary artery disease (see Figure VI in the online-only Data Supplement). This was controversially discussed in the past as some trials showed the association and some failed. We further strengthen the concept that hypertension (next to diabetes mellitus and smoking) is independently associated with psoriasis. This is in line with previous observations demonstrating that severe psoriasis is an independent risk factor.
factor for cardiovascular mortality besides other traditional cardiovascular risk factors and that psoriatic patients have a heavier burden of the development CVD events. In conclusion, our study indicates that (1) arterial hypertension and vascular disease is correlated with psoriasis-like skin disease in both mice and humans and that (2) the K14IL-17A mice represent a viable model to further study the mechanistic link between psoriasis-like skin inflammation and vascular inflammation/dysfunction in the absence of other cardiovascular risk factors.

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Disclosures

None.

References


27. Yilmaz SB, Cicek N, Coskun M, Yegin O, Alpsoy E. Serum and tissue interleukin-17 levels of TNF-α, IFN-gamma, IL-6, IL-8, IL-12, IL-17, and IL-18 in patients with active
The overexpression of IL-17A in the skin leads—besides evoking a psoriasis-like skin phenotype—to a systemic vascular dysfunction, increased oxidative stress, arterial hypertension, and increased mortality in mice. Neutrophil granulocytes triggered by IL-17A seem to play an important role in linking the skin and the vascular disease in our mouse model. It remains to be further elucidated whether these inflammatory mechanisms also play a role in humans, where psoriasis and vascular dysfunction have been described to be correlated for a long time. Neutralization of cytokines downstream of IL-17A by biologicals might be useful to treat the cardiovascular sequelae of severe psoriasis and offer possible new therapeutic options to treat vascular disease in psoriasis patients, although the multifunctional role of IL-17A in the development of atherosclerosis has to be kept in mind and further analysis has to follow.
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Interleukin 17 drives vascular inflammation, endothelial dysfunction and arterial hypertension in psoriasis-like skin disease

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# contributed equally

Supplementary Material and Methods

Mice

Animal treatment was in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the U.S. National Institutes of Health and was granted by the University Medical Center Mainz Ethics Committee.

The K14-IL-17Aind/+ mice1 were generated by crossing the IL-17Aind allele (previously described in2 and 1, resulting from genetically changed Bruce4-stemcells and backcrossed to C57Bl/6 over 50 generations) to the K14-Cre allele3. In K14-Cre mice, Cre-recombinase was shown to be expressed specifically in keratinocytes with low background levels in the
For breeding, only male mice were taken to transmit the Cre gene. In the K14-IL-17A\(^{\text{ind/+}}\) mouse strain of IL-17A (and EGFP, enhanced green fluorescent protein) is over-expressed in the skin based on Cre-mediated recombination. As control group IL-17A\(^{\text{ind/+}}\) mice were used. Usually mice of 3-5 months were used for experiments.

For endothelial IL-17A over-expression, the IL17-A\(^{\text{ind/ind}}\) mouse strain was crossed with the Tie2-Cre strain\(^4\) (Tie2-Cre-IL-17A\(^{\text{ind/+}}\)). For general IL-17A over-expression, the IL17-A\(^{\text{ind/ind}}\) mouse strain was crossed with the Deleter Cre strain\(^5\) resulting in the Del-Cre-IL-17A\(^{\text{ind/+}}\) strain. Also here, IL-17A\(^{\text{ind/+}}\) mice were used as control group.

For all breedings, only male mice were taken to transmit the Cre gene.

**Chemicals**

All chemicals were of analytical grade and ordered from either Fluka, Merck or Sigma.

**Scoring of psoriatic lesions in mice (psoriasis area and severity index = PASI)**

Skin lesions were scored using a modified score based on the human PASI score\(^6\) as described previously\(^1\) describing the degree of erythema, scaling of skin and skin thickness (0=no affection, 1=mild, 2=moderate, 3=severe, 4=very severe) and the percentage of affected skin referred to the total body surface. For the cumulative score, the sum of the first three parameters is multiplied with the percentage of the affected skin. Skin thickness is measured in duplicates by using a micrometer (Mitutoyo).

**Vascular relaxation studies of aortic rings**

The thoracic part of aortas isolated from IL-17A\(^{\text{ind/+}}\) and K14IL-17A\(^{\text{ind/+}}\) mice were liberated from fatty tissue and then cut into 4 mm segments. Then they were carefully rinsed to be completely free from blood inside the vessel. The endothelium-intact segments were put on force transducers (Kent scientific corporation, Torrington, USA; Powerlab, ADInstruments, Spechbach, Germany) in organ chambers filled with Krebs-Henseleit solution\(^7\) to perform concentration-relaxation curves of aortic tissue in response to increasing
concentrations of acetylcholine (ACh) and glyceryl trinitrate (GTN) as described previously.8

**Determination of blood pressure**

Systolic blood pressure was obtained over 3 weeks in mice using a tail cuff non-invasive blood pressure system coupled to a PowerLab system (ML125 NIBP, ADInstruments) along a protocol that was previously published9. A minimum of three measurements were obtained from each mouse per week, mice were measured over one month. The psoriasis-like skin affection did not allow us to successfully implant telemetric catheters as we usually do for blood pressure measurements7, so we had to switch to non-invasive blood pressure measurements.

**Quantification of Reactive Oxygen Species**

Oxidative burst of the whole blood and NADPH-oxidase activity of the cardiac membrane fraction was measured with lucigenin (5µM)- and L-012 (100µM)-enhanced chemiluminescence (ECL) as previously described10, 11:

Venous blood was taken from the heart, sodium citrate was added and the blood was kept at room temperature. The L-012 ECL signal was counted with PDBU (10 µM) and without (basal) and was expressed as counts per minute.

For analyzing the NADPH oxidase activity in the heart membrane fractions the membrane fractions were isolated by centrifugation up to 100,000 g for 60 minutes. After resuspension of the pellet the NADPH oxidase activity was measured by lucigenin (5 µM) ECL after adding 200 µM NADPH. The results were normalized along the protein content per sample. They were expressed as counts/mg/min.

Thoracic cryosections of aortas were stained with the superoxide-sensitive dye dihydroethidium (DHE, 1 µM) to perform fluorescence oxidative microtopography7:

After being rinsed and cleaned, the aorta was cut into 3 mm sections. They were incubated in Krebs-Henseleit-solution for 15 minutes at 37°C and then embedded in Tissue
Tec and frozen in liquid nitrogen. Aortic cryosection of 8 \( \mu \text{m} \) were cut, stained with DHE and incubated for 30 min at 37\(^\circ\)C. The green autofluorescence from aortic lamina and red ethidium fluorescence inside the ROS producing cells was detected by fluorescence light microscopy (Zeiss Axiovert 40 CFL microscope, Zeiss lenses and Axiocam MRm camera, Zeiss, Oberkochen, Germany) and analyzed with the Axio vision data acquisition software (Zeiss).

**Quantification of NO formation**

The amount of total NO synthesis as nitrite in serum was measured as total nitrite after enzymatic reduction of nitrate with nitrate reductase. Nitrite was identified in the serum by chemiluminescence after chemical reduction to NO with a NOA 280 Nitric Oxide Analyzer (Sievers) as described\(^{12}\).

**Quantitative Real-Time Reverse-Transcription Polymerase Chain Reaction (real-time RT-PCR)**

Total RNA was isolated from skin and aortic tissue using the RNeasy Mini Kit (Qiagen) after controlled crushing with Tissue Lyzer (Qiagen) and Proteinase K (Qiagen) incubation.

Real-time RT-PCR was performed either using one-step or two-step RT-PCR:

For one-step real-time RT-PCR 0.5\( \mu \text{g} \) of total RNA was used for analysis with the QuantiTect\textsuperscript{TM} Probe RT-PCR kit (Qiagen). A Taq-Man Gene Expression assay for the denoted primers was used as probe-and-primer set (Applied Biosystems).

For two-step real-time RT-PCR cDNA was prepared using the first strand synthesis kit from Invitrogen. One microgram of cDNA was used for a quantitative real-time reaction using the QuantiTect SYBR Green reaction mixture (Qiagen) on white 96-well plates (Roche) with primer mixes from Qiagen as described on their homepage.

The relative expression levels of the respective samples to HPRT, GAPDH or TATA-Box as endogenous control (housekeeping genes) were calculated with the delta-delta Ct method\(^{13}\).
**Western Blot Analysis**

Isolated aortic tissue was cleaned of fatty tissue, shock-frozen and homogenized in liquid nitrogen. The tissue homogenates were adjusted for protein content, separated by SDS-PAGE, blotted onto a nitrocellulose membrane and blocked. Immunoblotting (Biorad, Hercules, USA) was performed with antibodies against NOX2 (Transduction Laboratories, Lexington, USA), P-VASP (Upstate, Lake Placid, NY, USA) and Hemeoxygenase-1 (Stressgen, Victoria, Canada), β-actin (rabbit polyclonal, Sigma Aldrich, Seelze, Germany), alpha-actinin (rabbit polyclonal, Sigma-Aldrich, Seelze, Germany). Detection was performed by ECL with peroxidase conjugated anti-rabbit/mouse secondary antibodies (Vector Lab., Burlingame, CA). Immunodetection was fulfilled with ECL Reagent (Amersham, Piscataway, USA). Antibody-specific bands were finally quantified by densitometry.

**Flow Cytometry Analysis**

Complete aortic vessels were cleaned of fat tissue and incubated either in collagenase II (1 mg/ml) and DNase I (50 µg/ml)\(^{14}\) or Liberase TM (1mg/ml)\(^{15}\) (all Sigma) for 20 min at 37°C. Aortic vessels were passed through cell strainers after digestion. Back skin was cleaned of fat tissue and incubated in a Liberase (Roche) and DNase solution (Sigma-Aldrich) for 1-1.5 hours before finally shredding with gentleMacs Dissociator (Miltenyi Biotec) and passing through a cell strainer\(^{16}\). For FACS analysis of blood, the blood samples were treated with BD red blood cell lysis kit following the standard protocol. Cells were treated with Fc-block (eBioscience). Surface staining was performed with anti-CD11b, anti-GR-1, anti-F4/80, anti-B220, anti-CD3 and anti-CD4. All antibodies were coupled to FITC, PE, V450, PE-Cy7, APC-Cy7, PerCP, V500 or APC (eBioscience; BD; Pharmingen). Concerning the tissue treated with liberase or collagenase (aortas and skin), dead cells were excluded with the help of a dead-cell marker before performing the analysis. Flow cytometric analysis was performed with FlowJo software. For the aortas, total invading cells were calculated for 1cm of aorta.
Histology

For hematoxylin and eosin staining, the described organs were isolated from the experimental mice. Samples were fixed in 4% paraformaldehyde, paraffin-embedded, cut and stained with hematoxylin and eosin according to standard protocols.

For immunohistochemistry, thoracal aortic segments were paraffin embedded, cut and immunostained with primary antibodies for iNOS and nitrotyrosine. Following the species of primary mAb appropriate biotinylated secondary antibodies were used after dilution following the manufacturer’s instructions. For immunochemical detection ABC reagent (Vector) and then DAB (peroxidase substrate Kit, Vector) reagent as substrate were used.

Fluorescence immunohistochemistry of 10 μm cryosections of skin and aorta was performed using the fluorescence microscope Olympus IX81 and the TSA Cy3 and TSA Fluorescein system (Perkin Elmer) as recommended by the company. The following primary antibodies were used: F4/80 (BD Bioscience), MPO (Abcam), CD4 (BD), IL-17A (Santa Cruz Biotechnology). The slides were incubated for 30 min at room temperature with the biotinylated secondary antibody (Dianova). Nuclei were counterstained with Hoechst 3342 (Invitrogen).

In vivo imaging

In vivo imaging of mice was performed using a Maestro in vivo imager (Intas, Germany)\textsuperscript{17}. EGFP signal is shown as thermal gradient.

Statistical analysis of mouse data

Mouse data were analyzed for statistical significance with GraphPad Prism 5. The two-tailed unpaired student’s t-test, one-way ANOVA test with Bonferroni correction or the Friedman test with post hoc Dunn test was used as appropriate. The EC50 value for vascular relaxations studies was obtained by log-transformation. Columns in figures represent means
Supplemental Material and Methods

± SEM; p-values of <0.001, P<0.01, and P<0.05 were considered statistically significant and marked by three, two, and one asterisks, respectively.

Analysis of the burden of cardiovascular complications in psoriasis

Hospitalized psoriasis-patients of the Department of Dermatology, University Medical Center Mainz, (time of hospitalization between 2004 and 2011) aged 25 to 85 years (n=418 in total and n=125 for calculation of the Framingham CVD score), were retrospectively compared in a case-control setting to an age- and gender-matched population-based sample without psoriasis (n=1254 in total and n=375 for calculation of the Framingham CVD score) from the Gutenberg Health Study18.

For analysis, smoking was dichotomized into never smokers and ever smokers (former and current smokers). Body mass index was used as a marker of obesity with a cutpoint of ≥30kg/m2. Diabetes mellitus was defined as a diagnosis of diabetes by a physician or a blood glucose level of ≥126mg/dl at the baseline examination after an overnight fast of at least 8 hours, or a blood glucose level of ≥200mg/dl in the baseline examination after a fasting period <8 hours in the population-based sample and as treated with oral blood glucose lowering therapy or insulin in the psoriasis sample. Dyslipidemia was defined as a diagnosis of dyslipidemia by a physician, medical treatment for dyslipidemia or as an LDL/HDL-ratio of >3.5. Hypertension was defined as systolic blood pressure of ≥140mmHg or diastolic blood pressure of ≥90mmHg at rest or intake of antihypertensive drugs. A positive family history of myocardial infarction was defined as history of myocardial infarction in a female first-degree relative <65 years or in a male first-degree relative <60 years. In cases, information on diabetes was available in 338 patients only, obesity in 390, dyslipidemia in 301, myocardial infarction (MI) in 286, coronary artery disease (CAD) in 268, peripheral artery disease (PAD) in 282, stroke in 286 and kidney disease in 289 patients.

Values from controls were deleted if data were missing for the respective case. Data for cardiovascular risk factors and co-morbidities were expressed as mean ±SD and relative and absolute frequencies; p-value from fisher’s exact test.
To analyze the association of cardiovascular risk factors with psoriasis, the factors hypertension, diabetes, smoking, obesity and dyslipidemia were selected as independent variables and psoriasis as independent variable in a univariable and multivariable-adjusted conditional logistic regression model (CLR). For univariable modeling, CLR was performed for each risk factor separately. 95% confidence intervals (CI) are given for the estimated odds ratios; p-values for the model were from Wald’s-test.

The general 10-year risk for an event of cardiovascular disease (CVD) was calculated by the updated Framingham risk score. CVD risk was categorized in three risk groups: low risk <10%, medium risk 10%-20% and high risk >20%. Result from controls was deleted if data was missing for the respective case. Median and 25th/75th quantiles of the Framingham CVD risk score were calculated for cases and controls. P-value for difference was determined by usage of the U-test.
References for Supplemental Material and Methods


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¹Institute for Molecular Medicine, ²Department of Medicine 2, ³Department of Dermatology, ⁴Department of Pharmacology, ⁵Institute for Pathology, ⁶Center for Thrombosis and Hemostasis and ⁷German Center for Cardiovascular Research (DZHK), partner site RhineMain and ⁸Department of Medicine 3, University Medical Center of the Johannes-Gutenberg University of Mainz, Germany; ⁹Department of Internal Medicine I, University Hospital Erlangen, Germany; ¹⁰Institute of Anatomy, University of Leipzig, Germany; ¹¹Institute for Biochemistry, Christian-Albrechts-University of Kiel, Germany; ¹²Institute of Biochemistry and Molecular Biology II, Medical Faculty, Heinrich-Heine University, Düsseldorf, Germany.

# contributed equally

Supplemental Figures
Supplementary Figure I

a

b

Skin

IL-17A\textsuperscript{ind/+}

K14-IL-17A\textsuperscript{ind/+}

CD1b

FSC

GR1

F4/80

* F4/80+

0 1 2 3 4

total cells skin (X 10\textsuperscript{5})

CD11b+ GR1+ F480+

* IL-17A\textsuperscript{ind/+}

K14-IL-17A\textsuperscript{ind/+}

c

IL-17A\textsuperscript{ind/+}

K14-IL-17A\textsuperscript{ind/+}

4x

10x

20x
Local overexpression of IL-17A in the skin

**K14-IL-17A^ind/+** mice

**Dermato-immunological phenotype of the K14IL-17A^ind/+ mice**

- Acanthotically thickened epidermis
- Loss of the stratum granulosum
- Elongation of the papillary dermis
- Areas of hyper- and parakeratosis
- Multiple neutrophilic abscesses in the horny layer
- Dermal infiltration of CD11b^hi Ly6G^+ neutrophils and CD11b^+ F480^+ macrophages

**Predilection areas of the affected skin:** backskin, back of the head and legs

- Elevated levels of IL-6 (27 pg/ml +/- 5.6 versus 0 in controls) and IL-17A (4084 pg/ml +/- 253.4 versus 0 in controls)

**Extradermal comorbidities:** Arthritis and uveitis

- Elevated serum levels of IL-6 (27 pg/ml +/- 5.6 versus 0 in controls) and IL-17A (4084 pg/ml +/- 253.4 versus 0 in controls)

**Increase in the population of CD11b^+ GR1^+ granulocytes in the bone marrow**

**Reduced skin thickness and reduced numbers of skin infiltrating CD11b^+ cells under systemic anti-IL6 treatment**
Supplementary Figure II

a

IL-17A^{ind/+}  K14-IL-17A^{ind/+}  Tie2-IL-17A^{ind/+}

Aorta

b

Del-IL-17A^{ind/+}
K14-IL-17A^{ind/+}
IL-17A

Ear  Lung  Liver  Kidney  Heart
Supplementary Figure III

(a) Serum nitrite levels in IL-17Aind/+ and K14-IL-17Aind/+ mice, with no significant difference (ns).

(b) mRNA levels relative to HPRT in IL-17Aind/+ and K14-IL-17Aind/+ mice, with no significant difference (ns).

(c) Serum nitrite concentrations in IL-17Aind/+ and K14-IL-17Aind/+ mice, showing a significant difference (***).
Supplementary Figure V

K14-IL-17A<sup>ind/+</sup>

Colon

Small intestine

Liver

Spleen

Lung

Kidney

Thyroid Gland
Supplementary Figure VI

Predicted 10-year Risk for Cardiovascular Disease

<table>
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<th>Control Group (n=375)</th>
<th>Psoriasis Patients (n=125)</th>
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<td><strong>OR (95% CI) p-value</strong></td>
<td>1.7 (1.2, 2.4) 0.003</td>
<td>1.6 (1.2, 2.0) &lt;0.001</td>
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<td>3.6 (2.3, 5.5) &lt;0.001</td>
<td>4.2 (3.0, 6.0) &lt;0.001</td>
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<td>0.78 (0.53, 1.2) 0.216</td>
<td>1.2 (0.89, 1.7) 0.217</td>
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Cardiovascular Risk Profile in Psoriasis Patients

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<th>CV risk factors</th>
<th>Psoriasis Patients (N=418)</th>
<th>Control Group (N=1,254)</th>
<th>P for difference</th>
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Comorbidities

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<td>MI, % (n)</td>
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<td>CAD, % (n)</td>
<td>17.9% (48) &lt;0.0001</td>
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<td>PAD, % (n)</td>
<td>5.32% (15) 0.88</td>
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<tr>
<td>Stroke, % (n)</td>
<td>7.34% (21) 0.00091</td>
</tr>
<tr>
<td>Kidney disease,% (n)</td>
<td>10.0% (29) &lt;0.0001</td>
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Supplemental Figure Legends

Supplementary Figure I  (a) 8 weeks old K14-IL-17A\textsuperscript{ind/+} mouse showing the described psoriasis-like phenotype. In comparison the healthy IL-17A\textsuperscript{ind/+} control mouse is shown. (b) Single cell suspensions obtained from mechanically disrupted skin of the same size (1.5 x 1.5cm) of K14-IL-17A\textsuperscript{ind/+} and control mice, treated with liberase/DNAse for digestion as described in the method’s section, were stained for CD11b, F4/80 and GR1 after having excluded the dead cells with a dead cell marker. Statistics for the individual populations are given on the right hand’s side (n=6-8 mice, student’s t-test). (c) Hematoxylin and eosin histology on skin sections of K14-IL-17A\textsuperscript{ind/+} mice compared to healthy control mice with focus on the skin vessels (n=6-7 mice). (d) Overview of the basic phenotypic characteristics of the K14-IL-17A\textsuperscript{ind/+} mouse model with respect to its immunological and dermatological features as previously published in \textsuperscript{1}.

Supplementary Figure II  (a) and (b) Indicated organs from Tie2-Cre-IL-17A\textsuperscript{ind/+}, Deleter Cre-IL-17A\textsuperscript{ind/+}, K14-IL-17A\textsuperscript{ind/+} and IL-17A\textsuperscript{ind/+} mice were analyzed by \textit{in vivo} imaging (EGFP signal shown as thermal gradient): The K14-Cre-recombinase is known to be specifically expressed in keratinocytes with low background levels in the thymus\textsuperscript{2}. To exclude unspecific expression and activity of the Cre-recombinase, the aortas of K14-IL-17A\textsuperscript{ind/+} mice were analyzed for EGFP signal as EGFP and IL-17A are colocalized after Cre-mediated recombination: No EGFP signal was detectable in the aortas or in any other organs of K14-IL-17A\textsuperscript{ind/+} mice except for the skin. A strong signal was observed in the aortas when IL-17A\textsuperscript{ind/+} was crossed to the Tie2-Cre\textsuperscript{3} and a systemic expression when crossed to a deleter-Cre strain (Del-IL-17A\textsuperscript{ind/+}).

Supplementary Figure III  (a) TNF-\alpha levels in the serum (measured by ELISA) and (b) expression level of TNF-\alpha in the skin (measured by real-time rt-PCR) of K14-IL-17A\textsuperscript{ind/+} compared to control mice. TNF-\alpha serum levels is shown as pg/ml and expression level in the
skin is shown relative to the housekeeping gene HPRT. (n=21-30 mice (ELISA) and n=5-10 mice (rt-PCR), both student’s t-test) (c) The amount of total NO synthesis in serum of the indicated mice was measured as total nitrite after enzymatic reduction of nitrate with nitrate reductase using a nitric oxide analyzer (n=16 mice per group). Student’s t-test.

Supplementary Figure IV  (a to d) Blood of K14-IL-17A\textsuperscript{ind/+} mice and IL-17A\textsuperscript{ind/+} controls was analyzed in the central laboratory of the university hospital Mainz for (a) creatinine, sodium, potassium and urea (kidney), (b) alanine-amino-transferase (ALAT), asparate-amino-transferase (ASAT), bilirubin and albumine (liver), (c) for lipase, amylase and calcium (pancreas), as well for (d) high-density lipoprotein (HDL), low-density lipoprotein (LDL), total cholesterol and blood glucose (fat and glucose metabolism). Student’s t-test.

Supplementary Figure V  Hematoxylin and eosin stainings (10x and 20x) of colon, small intestine, liver, spleen, lung, kidney and thyroid gland cuts of K14-IL-17A\textsuperscript{ind/+} mice. Besides large splenic cells and discrete signs for an interstitial lung infection, there are now further peculiarities apparent. (Representative pictures of n = 3 mice shown.)

Supplementary Figure VI  Psoriasis patients have a higher prevalence of cardiovascular disease: A case-control study was performed to compare the cardiovascular risk profile between psoriasis patients and a population-based sample free of psoriasis: Hospitalized psoriasis patients of the Department of Dermatology, University Medical Center Mainz were compared in a case-control setting to an age- and gender-matched population-based sample without psoriasis from the Gutenberg Health Study\textsuperscript{4} concerning their cardiovascular risk profile. (a) General 10-year risk for an event of cardiovascular disease (CVD), calculated by the updated Framingham risk score\textsuperscript{5}. CVD risk was categorized in low risk (<10%), medium risk (10%-20%) and high risk (>20%). Median and 25th/75th quantiles are given for each risk group. P for difference from U-Test. (n=125 psoriasis patients and n=375 healthy controls) (b) MI (myocardial infarction), CAD (coronary artery disease), PAD (peripheral arterial
disease). Data are expressed as mean ± SD and relative and absolute frequencies; p-value from fisher’s exact test. (n=418 psoriasis patients and n=1254 matched healthy controls) (c) Univariable and multivariable-adjusted conditional logistic regression model. For univariable modeling, CLR was performed for each risk factor separately; for multivariable modeling, the model was adjusted for all risk factors. 95% confidence intervals are given for the estimated odds ratios; p-values for model from Wald-test. (n=418 psoriasis patients and n=1254 matched healthy controls)
References for Supplemental Figures


