



Regulation of the Immune Cell Repertoire in Psoriasis Patients Upon Blockade of IL-17A or TNF α

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ABSTRACT

Introduction: Targeting of the proinflammatory cytokine interleukin 17A (IL-17A) or tumor necrosis factor alpha (TNF α) with the monoclonal antibodies (mAbs) ixekizumab or adalimumab, respectively, is a successful therapy for chronic plaque psoriasis. The effects of these treatments on immune cell populations in the skin are largely unknown.

Methods: In this study, we compared the composition of cutaneous, lesional and non-lesional immune cells and blood immune cells

in ixekizumab- or adalimumab-treated patients with psoriasis.

Results: Our data reveal that both treatments efficiently downregulate T cells, macrophages and different subsets of dendritic cells (DCs) in lesional skin towards levels of healthy skin. In contrast to lesional skin, non-lesional areas in patients harbor only few or no detectable DCs compared to the skin of healthy subjects. Treatment with neither ixekizumab nor adalimumab reversed this DC imbalance in non-lesional skin of psoriatic patients.

Conclusion: Our study shows that anti-IL-17A and anti-TNF α therapy rebalances the immune cell repertoire of lesional skin in psoriatic patients but fails to restore the disturbed immune cell repertoire in non-lesional skin.

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Key Summary Points

Why carry out this study?

Chronic plaque psoriasis is a common inflammatory skin disease. Monoclonal antibody therapy is the most effective treatment in moderate-to-severe cases

Our study compares the impact of two prominent biologics (ixekizumab and adalimumab) on the immune cell occurrence in the skin and blood

What was learned from the study?

Anti-IL-17A and anti-TNF α both regulate the increase in immune cells in diseased skin but are not capable of establishing a sound immune cell profile in uninvolved skin of psoriatic patients, as in healthy persons

The striking difference in the profile of DCs, especially the low frequency in non-lesional psoriatic skin, suggests that DCs could be involved in the localized onset and distribution of this systemic disease

Only ixekizumab was able to downmodulate the higher numbers of CD15⁺CD14⁻ cells in the blood of patients suffering from psoriasis and might therefore play a role in the differentiation of granulocytic myeloid-derived suppressor cells (GMDSCs)

INTRODUCTION

Chronic plaque psoriasis is an inflammatory disease with characteristic skin manifestations and affects 2–3% of individuals in Western countries. The disease burden is considerable, and the risk for comorbidities, including cardiometabolic or mental health disorders, is

increased within the psoriatic population but might be reduced by effective treatment [1–3]. Psoriasis is understood to be primarily a DC/T cell-driven disease [4–7]. The prevailing opinion is that epidermal keratinocytes hyperproliferate in response to the massive production of IL-17A by pathogenic CD4⁺ T-helper 17 cells (Th17) [8–11]. Th17 expansion in turn is induced by IL-23, which is released by activated myeloid DCs [12, 13]. However, IL-17A is also released by other cells in psoriatic skin, including polymorphonuclear neutrophils (PMNs) and mast cells [14–17]. The role of these cells in the pathophysiology of psoriasis is poorly defined. TNF α is released by several immune cells, including macrophages/monocytes, natural killer cells (NKs) and DCs [18–20]. In psoriasis, elevated TNF α stimulates DCs to produce IL-23 and additionally upregulates the proinflammatory scenery for keratinocytes in synergy with IL-17A [5, 21, 22]. While the knowledge of different immune cell types, particularly T cells and DCs, is profound in lesional skin, less is known about non-lesional skin or the peripheral blood in chronic plaque psoriasis patients [23–29]. In the study presented here, we analyzed and compared the blockade of IL-17A with TNF α inhibition on the immune cell profile in the skin and blood of psoriasis patients

METHODS

Study Design

This was an experimental ex vivo human study conducted at the Department of Dermatology and the Institute of Immunology of the Medical University of Vienna (Vienna, Austria). We investigated the impact of ixekizumab (IXE) and adalimumab (ADA) treatment on skin and blood samples from psoriatic patients. The study was approved by the ethics committee of the Medical University of Vienna (ECS 1642/2017) in accordance with the Helsinki Declaration of 1964 and its later amendments and followed national law and ethical principles in research. It required voluntary participation of patients and healthy controls with written informed consent. Study participation involved

the collection of necessary personal data, photography of involved skin areas and skin and blood samples. All patients gave written informed consent to the release of clinical images captured. The individual risk for participants was low, and adverse effects were not observed in the course of the study.

Recruitment of Subjects

Psoriasis patients from the outpatient clinic of the Department of Dermatology, Medical University of Vienna, as well as healthy controls were voluntarily enrolled in the study. All study participants were ≥ 18 and < 80 years of age. All included patients had stable (> 6 months) moderate-to-severe chronic plaque psoriasis involving $\geq 10\%$ of total body surface area (BSA), with a psoriasis area and severity index (PASI) score of ≥ 10 and a Physician Global Assessment (PGA) of ≥ 3 before treatment initiation. Biopsies and blood samples of subjects on therapy with either IXE or ADA were realized within 12 weeks after treatment initiation. Further details on the inclusion and exclusion criteria are listed in the supplementary material.

Collection of Samples and Staining Strategy

Clinical pictures of participants with psoriasis were made at the timepoint of inclusion (Figure S5). In each participant, blood and skin samples were collected. Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples. Six-millimeter (mm) punch biopsies were obtained in duplicate from lesional and non-lesional skin of psoriatic patients, whereas one 6-mm punch biopsy of healthy skin was obtained from controls. Biopsies were split into paraffin and frozen sections. Paraffin-embedded samples were used for histology (Figure S6), immunohistochemistry and archiving, whereas frozen samples were used for immunofluorescence (IF) staining. Based on a set of mAbs, multiplex immunofluorescence (mIF) coexpression analyses were established on acetone-fixed cryosections of lesional psoriatic, non-lesional psoriatic and healthy skin samples,

according to a mIF panel, particularly elaborated for this study (Table S2, S3; Figure S7, S8). A detailed description of sample collection and immunohistochemical and immunofluorescence staining protocols are disclosed in the supplementary material section.

Analysis of Skin Samples via TissueFAXS and TissueQuest

Stained skin tissue sections were acquired using the TissueFAXS imaging system (TissueGnostics GmbH, Vienna, Austria) on an Axio Observer Z1 microscope equipped with an LD Plan-Neofluar $20 \times /0.4$ objective (Zeiss). Data from the acquired photos were processed with TissueQuest image analysis software 6.0 and StrataQuest (TissueGnostics GmbH). Matched isotype controls were included for analysis of background staining. Biopsy specimens were read in a blinded fashion by an independent investigator who was not involved in the collection or staining of the tissue sections. The epidermis and dermis were analyzed separately. In each biopsy, an area of 5 mm^2 of the upper dermis and the epidermis (at a length of 5 mm basement membrane) was tagged. Labeled cells within the tagged area were expressed as the number of cells/ mm^2 . Additionally, the percentage and the absolute numbers of labeled cells within the selected area were also gathered. Only cells clearly positive for the antigen were counted. Artifacts, blood vessels and adnexa were excluded from the areas of interest.

Flow Cytometry

Flow cytometric analysis of single-cell suspensions was performed by surface marker staining. PBMCs (2×10^5) were incubated with conjugated mAbs (Table S2) for 30 min at 4°C . After washing with PBS-BSA, the cells were analyzed by flow cytometry (LSRFortessa™ Cell Analyzer, Becton Dickinson, Franklin Lakes, NJ).

Statistical Analysis

Flow cytometric data were analyzed using FlowJo data analysis software (Becton

Dickinson). Further statistical analysis for flow cytometric data and TissueQuest-generated output was performed via GraphPad Prism version 7.00 (GraphPad Software, La Jolla, CA). Therefore, the normality of the data distribution was assessed by applying the Shapiro-Wilk test. For a normal distribution, parametric one- or two-way analysis of variance (ANOVA) was performed, depending on the number of tested conditions. This was followed by Tukey's pairwise comparisons (Tukey's post hoc test) with adjusted *P* values. If a Gaussian distribution was not assumed, the nonparametric Kruskal-Wallis test was applied, followed by Dunn's correction for multiple comparisons. Probability values of $P < 0.05$ were considered statistically significant, represented as $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ and $****P < 0.0001$. The results are depicted as the mean \pm standard deviation (SD) in scatter plots or bar charts.

RESULTS

Study Population

We enrolled 21 subjects in this study divided into 4 subgroups: 5 healthy controls, 6 untreated psoriasis patients, 5 psoriatic patients under IL-17A inhibition with IXE and 5 individuals under TNF α blockade with ADA within the first 12 weeks of therapy. All groups showed comparable demographic characteristics (Table S1). All participants receiving mAb therapy responded to treatment with at least a PASI75% reduction within the first 6 months. The results presented in Fig. 1 demonstrate a mean PASI reduction of 94% and 87% among IXE- and ADA-treated subjects, respectively, indicating that all patients initially responded to treatment, excluding nonresponders. Further details on the study population, including respective body sites for biopsies, additional scores for disease activity (BSA, PGA), quality of life [Dermatology Life Quality Index (DLQI)] and the histopathologic psoriasis severity score (HPSS) at inclusion, are reported in the supplementary material.

Treatment of Psoriasis Patients with Ixekizumab or Adalimumab Reduces T Cells and NK Cells in the Skin

The results of our study demonstrate that CD4⁺ and CD8⁺ T cells were found at a higher density in lesional areas of the epidermal and dermal regions of the skin of untreated psoriasis patients than in healthy donors (Fig. 2). Treatment with IXE and ADA reduced the number of CD4⁺ T cells (Fig. 2a, b) and CD8⁺ T cells (Fig. 2d, e) in lesional skin to levels observed in healthy donors. An unexpected finding was that the amounts of CD3⁺Ki67⁺ T cells in the epidermis of patients treated with ADA were increased (Fig. S2a). The number and

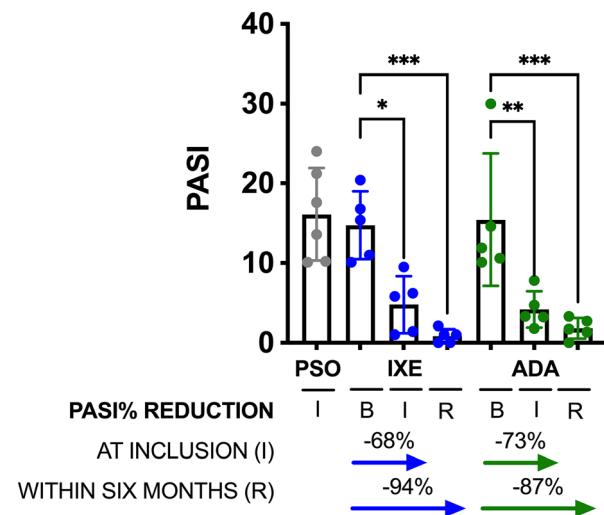


Fig. 1 Dynamics of the psoriasis area and severity index (PASI) upon treatment with IL-17A or TNF α inhibitors. Bar charts are depicted as the mean \pm standard deviation (SD) with respective single values plotted as points. Psoriatic patients were grouped into subpopulations based on their treatment regimen. PASI scores are shown at study inclusion (I) for all subpopulations. PASI scores under anti-IL-17A (IXE) or anti-TNF α (ADA) therapy are given before treatment initiation (B), at inclusion (I) and within 6 months (R) to confirm treatment response. The percent decrease in the baseline PASI at the timepoint of inclusion and within 6 months are presented for both treatment regimens separately. *C* Healthy control; *PSO* psoriasis without treatment; *IXE* psoriasis under IL-17A inhibition with ixekizumab; *ADA* psoriasis under TNF α inhibition with adalimumab; *ns* nonsignificant

percentage of T cells in the peripheral blood of psoriasis patients were not altered compared to those in healthy donors and were not significantly modulated upon treatment of patients with IXE or ADA (Fig. 2c, f). However, T cells isolated from the blood of psoriasis patients expressed higher levels of CD71 (Fig. S2c). The expression of T cell activation marker was significantly lower in patients treated with IXE or ADA.

Furthermore, untreated psoriatic plaques appeared with more epidermal CD56⁺ NK cells than healthy skin (Fig. S3a). In the healthy controls, only a few NK cells were present in the epidermis. An increase in epidermal NK cells was not detected under treatment with IXE and ADA (Fig. S3a). Interestingly, the number of dermal NK cells was only reduced by IXE but not upon ADA therapy (Fig. S3b). No relevant changes in CD19⁺ B cell counts were observed in the skin or blood within the different patient cohorts (Fig. S3d, e, f).

Impact of Treatment with Adalimumab or Ixekizumab on Low-Density CD15⁺CD14⁻ Cells

Granulocytes (CD15⁺CD14⁻) were rare in lesional and non-lesional areas of the dermis in psoriatic patients, and the density and frequency of these cells were not regulated by IXE and ADA treatment (Fig. 3b). In contrast, the percentage of granulocytes was significantly increased in the lesional epidermis of psoriatic patients (Fig. 3a) but not in the non-lesional epidermis (data not shown). Treatment with IXE or ADA reduced granulocytes in the epidermis to baseline levels of healthy controls (Fig. 3a). Analysis of the PBMCs by flow cytometry revealed an increased number of low-density CD15⁺CD14⁻ cells, potentially resembling granulocytic myeloid-derived suppressor cells (GMDSCs), in patients with psoriasis compared to healthy controls. Interestingly, CD15⁺CD14⁻ cells were downregulated in IXE- but not ADA-treated patients within the induction phase of therapy (Fig. 3c, d). Thus, ADA and IXE differ in their capacity to

downregulate CD15⁺CD14⁻ cells in psoriasis patients.

Treatment of Psoriasis Patients with Ixekizumab or Adalimumab Does Not Restore the Repertoire of DCs in the Skin

Langerhans cells (LCs) are prototypic CD1a⁺ cells in the epidermis. The density of LCs in lesional but not in non-lesional areas of psoriatic skin was found to be significantly decreased (Fig. 4a, b). Neither treatment with IXE nor treatment with ADA changed LC numbers in the psoriatic epidermis. In contrast to epidermal LCs, the density of CD1a⁺ cells was strongly increased in the dermis of lesional skin (Fig. 4c). CD1a⁺ cells, which could resemble inflammatory DCs, were absent in the dermis of healthy controls as well as in the non-lesional dermis of psoriasis patients (Fig. 4d). A high number of CD1a⁺ cells in the lesional dermis was not detected in patients upon IXE or ADA treatment (Fig. 4c). Elevated percentages of CD1a⁺ cells were also detected in the blood of psoriasis patients, which were normalized to levels in healthy controls upon both treatment regimens (Fig. 4e). Other characteristic markers of DCs that were analyzed in this study were CD141, which is expressed on conventional DC-type 1 (cDC1), and CD303, a marker for plasmacytoid DCs (pDCs) (Fig. 4) [30–33]. The density of CD141⁺ and CD303⁺ cells was significantly decreased in non-lesional zones of the dermis of psoriasis patients compared to healthy skin (Fig. 4i, n). This cellular profile was not restored in patients upon treatment with IXE or ADA. The results presented in Figure S4 demonstrate that in contrast to DCs, the levels of CD68⁺ cells, which are most likely macrophages, in non-lesional skin were similar to those in healthy controls.

The lesional skin of psoriasis patients harbored significantly more CD141⁺ and CD303⁺ cells than that of healthy controls (Fig. 4h, m). Here, treatment with IXE or ADA reversed the numbers of both cell types to levels of healthy controls (Fig. 4h, m). In the blood, the numbers of CD303⁺ cells were increased in psoriasis

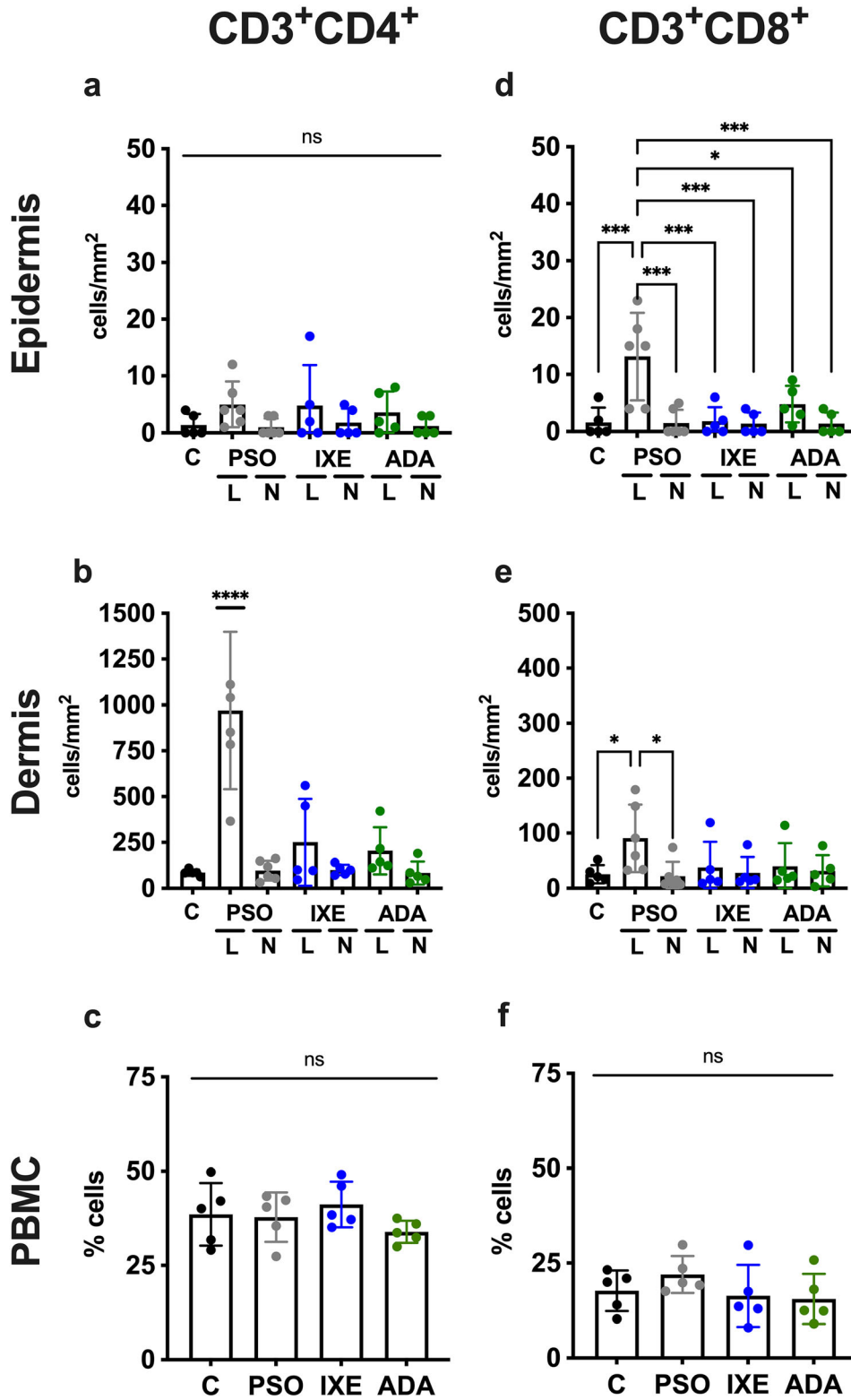


Fig. 2 Impact of ixekizumab and adalimumab treatment on T cells in psoriatic skin and blood. Epidermal and dermal CD3⁺ T cells, including their combination with CD4⁺ (a, b) and CD8⁺ (d, e) cells, are given in numbers/mm² as bar charts with the mean ± standard deviation (SD) and respective single values plotted as points. The corresponding CD3⁺ populations in the blood are equally depicted in % of PBMC fraction (c, f). Psoriatic patients were grouped based on their treatment regimen during the induction phase. C Healthy control; PSO psoriasis without treatment; IXE psoriasis under IL-17A inhibition with ixekizumab; ADA psoriasis under TNFα inhibition with adalimumab; ns nonsignificant; L lesional; N non-lesional

patients, and expression was absent upon IXE but not adalimumab therapy (Fig. 4o). The expression of CD141⁺ cells in the blood was similar in psoriatic patients and healthy donors (Fig. 4j). Taken together, IXE and ADA had a comparable impact on the DC compartment of psoriatic patients but did not restore the repertoire of DCs in non-lesional skin or LC in the epidermis.

DISCUSSION

The biologicals IXE directed against IL-17A and ADA against TNFα have significantly improved

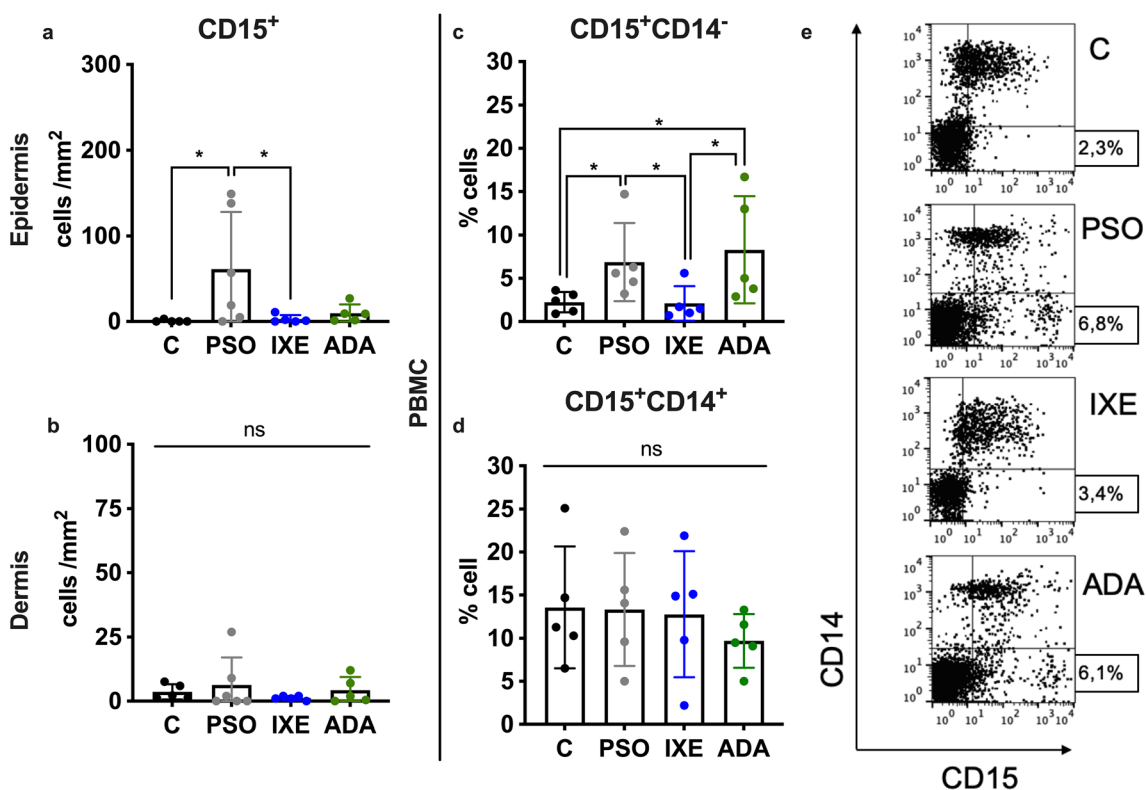


Fig. 3 Changes in the granulocyte cell system upon IL-17A and TNFα inhibition. Expression of CD15⁺ granulocytes in lesional skin (a, b), and the PBMC fraction (c, d) of psoriatic patients was analyzed. Data are depicted in either cells/mm² (skin) or % (PBMCs) as bar charts with the mean ± standard deviation (SD). Single values are plotted as points. Psoriatic patients were grouped into subpopulations based on their treatment regimen during the induction phase. A representative flow cytometry plot

of CD15⁺CD14⁻ cells in a healthy control, an untreated psoriatic patient and a patient under anti-IL-17A (IXE) or anti-TNFα (ADA) therapy is shown under e. Percentages of CD15⁺CD14⁻ cells are displayed on the right. C Healthy control; PSO psoriasis without treatment; IXE psoriasis under IL-17A inhibition with ixekizumab; ADA psoriasis under TNFα inhibition with adalimumab; ns nonsignificant

◀**Fig. 4** Dynamics of dendritic cells in lesional versus non-lesional skin and blood in response to anti-IL-17A or anti-TNF α therapy. Bar charts are depicted as the mean \pm standard deviation (SD), including lesional and non-lesional epidermal and dermal CD1a $^{+}$ (a–d), CD141 $^{+}$ (f–i) and CD303 $^{+}$ (k–n) cells in numbers/mm 2 . Corresponding CD1a $^{+}$ (e), CD141 $^{+}$ (j) and CD303 $^{+}$ (o) cells of the PBMC fraction are given in %. Single values are plotted as points. Psoriatic patients were grouped into subpopulations based on their treatment regimen during the induction phase. *C* Healthy control; *PSO* psoriasis without treatment; *IXE* psoriasis under IL-17A inhibition with ixekizumab; *ADA* psoriasis under TNF α inhibition with adalimumab; *ns* nonsignificant

the handling of moderate to severe psoriasis [34]. In this study, we performed a head-to-head analysis of immune cell distribution and frequency in lesional and non-lesional skin and blood of IXE- and ADA-treated patients with psoriasis. Our data show that both treatment strategies have similar effectiveness in reducing the numbers of key drivers of the disease—T cells, DCs and pDCs—in lesional areas of psoriatic skin. In addition, IXE- but not ADA-treated psoriatic patients exhibited downregulated NK cells in lesional regions of the skin and low-density CD15 $^{+}$ CD14 $^{-}$ cells in the blood. The effective downregulation of immune cells in diseased skin, demonstrated with anti-IL-17A and anti-TNF α in this study, raises the question of whether treatment with these monoclonal antibodies can decrease inflammatory damage in other tissues apart from the skin. A protective scenario in pre-clinical enthesitis was already shown for patients with psoriasis under adalimumab [35]. Studies on anti-IL-17A agents, also report promising results for patients with psoriatic arthritis [36, 37]. Future studies on the immune cell profile under treatment in tissues involved in psoriasis-associated comorbidities are crucial to identify if the effects are similar to the skin. To be able to decrease tissue damage or even prevent psoriatic comorbidities such as psychiatric, cardiovascular or arthritic involvement would justify early systemic treatment and minimize disease burden.

Psoriasis is typically accompanied by an increase in different types of DCs, in particular

CD1a $^{+}$, cDC1 and pDCs, in the lesional dermis [21, 38–42]. Interestingly, we discovered that the number of DCs in the non-lesional skin was significantly lower, even compared to healthy donors (Fig. 4). The striking difference in the profile of DCs in psoriatic skin suggests that DCs could be involved in the localized onset and distribution of this systemic disease. The auto-induction of psoriatic lesions in xenografts of unaffected psoriatic skin of immune-deficient mice supposed a regulatory assignment of resident immune cells and local immune environments [43]. In this model, pDCs were rapidly recruited to the xenograft and played an important role during the initiation phase of the psoriatic plaque formation [21] by producing type I interferon and promoting functional maturation of myeloid DCs in psoriasis [44, 45]. Thus, the depletion of pDCs in non-lesional psoriatic skin demonstrated in our results might be required to keep the respective skin unaffected in a patient with psoriasis. However, the consequences of the low frequency of DCs in non-lesional psoriatic skin need to be studied in more detail in future studies. The results of our study further demonstrate that both ADA and IXE treatment regimens did not restore the low amounts of DCs in the dermis or LCs in the epidermis in non-lesional or lesional areas of psoriatic skin, respectively. Thus, both biologicals are effective in regulating the increase in immune cells in diseased skin but are seemingly not capable of establishing a sound immune cell profile in uninvolved skin of psoriatic patients as in healthy persons.

The number of CD68 $^{+}$ cells was reduced because of IXE or ADA treatment, suggesting that the increase in macrophages is controlled by both biologicals. In contrast to DCs, the number of CD68 $^{+}$ cells in non-lesional skin was comparable to that in the skin of healthy individuals and did not point to a disturbed immune balance in the unaffected skin of psoriasis patients.

Another unexpected finding was the elevated number of low-density CD15 $^{+}$ CD14 $^{-}$ cells in the PBMC fraction of psoriasis patients, which could resemble GMDSCs. GMDSCs, also described as polymorphonuclear-myeloid-derived suppressor cells (PMN-MDSCs), are a

relatively new subset of regulatory myeloid-derived cells of the phagocytic cell system that are generated under inflammatory conditions and inhibit effector T cells [46–49]. GMDSCs have been detected in various diseases, above all in cancer but also in sepsis or autoimmune disease [50–56]. Although myeloid suppressor cells in general have shown a remarkable ability to suppress T cell responses in cancer, their role seems to be more heterogeneous in autoimmune diseases such as psoriasis [29, 57]. It has been reported that GMDSCs are elevated in the blood of psoriatic patients [58], which could be interpreted as a compensatory mechanism in chronic inflammatory diseases. However, IXE but not ADA was able to downmodulate the higher numbers of CD15⁺CD14⁻ cells in patients suffering from psoriasis. The fact that GMDSCs in vitro promoted Th17 differentiation matches the observation that anti-IL-17A but not anti-TNF α downmodulated the higher numbers of these cells in the blood of our patients [59]. Thus, IL-17A but not TNF α is seemingly a key factor for the differentiation of GMDSCs in the human immune system. It remains to be determined in future studies whether the CD15⁺CD14⁻ cells that we found in the PBMCs of our patients are indeed GMDSCs [50, 60, 61]. If so, the presence or absence of GMDSCs before and after IXE therapy could be used as a biomarker to monitor disease progression and therapeutic success.

The mechanisms underlying the treatment of patients with ADA and IXE have been studied and discussed in many studies [62–66]. We observed that neither of the two biologicals induced enhanced apoptosis in immune cells in either the skin or the blood. However, we detected reduced expression of activation markers on T cells, suggesting that inflammatory signals stemming from inflamed tissue may promote T cell activation and probably also T cell proliferation, which is supported by the finding of increased levels of CD71 on T cells in psoriatic patients and the inhibition of CD71 by IXE and ADA.

A limitation of our study was the relatively small study collective of 21 subjects. Biopsies are not a standard procedure at the Medical University of Vienna in the monitoring of

psoriasis patients. So, the number of patients included in the study was intended to be small. Additionally, participants were enrolled according to their current treatment status and were not prospectively followed up. Nevertheless, data between patients were generally consistent, arguing against major interpatient variability. Novel methods such as single-cell RNA sequencing (scRNAseq) have significantly advanced our knowledge in the field and led to the description of additional cell types [67–73]. The findings of expanded T/NK cells in lesional psoriatic skin corroborate recent transcriptome studies, which deployed scRNAseq to resolve the cellular heterogeneity of inflammatory skin and confirmed a Tc17/Th17 signature in psoriasis [72–75]. The increased numbers of dendritic cells in lesional psoriatic skin in our study are a confirmatory finding and in accordance with flow or transcriptome analyses [69, 72]. The low frequency of DCs in non-lesional psoriatic skin demonstrated by our results are unexpected and in a way contrary to previous reports [71]. Follow-up single-cell analyses might focus on non-lesional psoriatic skin compared to healthy and lesional psoriatic skin to further elucidate the gene programs involved in the localized onset of the disease. In summary, the key aspects of the immune cell profile in psoriasis correspond between multiplex immunohistochemistry/immunofluorescence techniques and transcriptome analysis, including scRNAseq. The purpose of our study was, however, not to better characterize immune cells and their subsets in psoriatic skin but to compare the impact of two prominent biologicals (IXE, ADA) on the immune cell occurrence.

CONCLUSION

Taken together, the results of our study demonstrate that ADA and IXE are both effective in downregulating the increase in prominent cellular drivers of psoriasis but fail to revert the DC deficiencies in non-lesional parts of the skin.

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Data Availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author (Julia Tittes) on reasonable request.

Declarations

Conflict of Interest. Other than that mentioned above, Julia Tittes reports consulting fees from Lilly, speaker honoraria from Lilly and Novartis, all outside the submitted work, as well as travel support from Almirall, AbbVie and Bristol-Myers Squibb through institution. Julia M. Ressler received speaker honoraria from Bristol-Myers Squibb, Roche, Amgen and Novartis and travel support by Sanofi, Roche and Bristol-Myers Squibb through institution. All other authors declare no conflicts of interest (COI). The corresponding author, Julia Tittes, has changed her affiliation after the completion of the manuscript. Her new affiliation is the Institute for Clinical Pathology and Molecular Pathology at the University Clinic St.Pölten-Lilienfeld (Karl Landsteiner University).

Ethical Approval. The study was approved in accordance with the Helsinki Declaration of 1964 and its later amendments by the ethics committee of the Medical University of Vienna (ECS 1642/2017) on the 25th of September 2017 and followed national law and ethical principles in research. It required voluntary participation of patients and healthy controls with written informed consent. Additionally, all patients gave written informed consent to the release of clinical images captured.

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