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Sebocytes contribute to skin inflammation by promoting the differentiation of T helper 17 cells*

M. Mattii,[1] M. Lovászi,[2] N. Garzorz,[3] A. Atenhan,[1] M. Quaranta,[1] F. Lauffer,[3] A. Konstantinow,[3] M. Küpper,[1] C.C. Zouboulis,[4] L. Kemeny,[5] K. Eyerich,[3] C.B. Schmidt-Weber,[1] D. Törőcsik[2] and S. Eyerich[1]

[1]ZAUM – Center for Allergy and Environment, Technische Universität and Helmholtz Center Munich, Member of the German Center for Lung Research (DZL), Biedersteinerstraße 29, 80802 Munich, Germany
[2]Department of Dermatology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary
[3]Department of Dermatology and Allergy, Technische Universität Munich, Munich, Germany
[4]Departments of Dermatology, Venereology, Allergology and Immunology, Dessau Medical Center, Dessau, Germany
[5]Department of Dermatology and Allergology, University of Szeged, Szeged, Hungary

Summary

Background The main function of sebocytes is considered to be the production of lipids to moisturize the skin. However, it recently became apparent that sebocytes release chemokines and cytokines and respond to proinflammatory stimuli as well as the presence of bacteria.
Objectives To analyse the functional communication between human sebocytes and T cells.
Methods Immunofluorescence stainings for CD4 and interleukin (IL)-17 were performed on acne sections and healthy skin. Migration assays and T-cell-stimulation cultures were performed with supernatants derived from unstimulated or prestimulated SZ95 sebocytes. Dendritic cells were generated in the presence of SZ95 supernatant and subsequently used in mixed leucocyte reactions.
Results We showed that $CD4^+$ $IL\text{-}17^+$ T cells accumulate around the pilosebaceous unit and are in close contact with sebocytes in acne lesions. By using SZ95 sebocyte supernatant, we demonstrate a chemotactic effect of sebocytes on neutrophils, monocytes and T cells in a CXCL8-dependent manner. Furthermore, sebocyte supernatant induces the differentiation of $CD4^+$ $CD45RA^+$ naive T cells into T helper (Th)17 cells via the secretion of IL-6, transforming growth factor-β and, most importantly, IL-1β. No direct effects of sebocytes on the function of $CD4^+$ $CD45RO^+$ memory T cells were detected. Moreover, sebocytes functionally interact with *Propionibacterium acnes* in the maturation of dendritic cells, leading to antigen-presenting cells that preferentially prime Th17 cells.
Conclusions Our study provides evidence that human sebocytes actively participate in inflammatory processes in the skin by recruiting and communicating with immune cells. This interaction leads to the generation of Th17 cells, which might contribute to the pathogenesis not only of acne vulgaris, but also of several inflammatory skin diseases.

What's already known about this topic?

- Sebocytes are part of the pilosebaceous unit and produce lipids for moisturizing the skin.
- They were long regarded as bystander cells during skin inflammation with no impact on the immune response.

Correspondence
Stefanie Eyerich.
E-mail: Stefanie.eyerich@tum.de

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Conflicts of interest
None declared.

M.M. and M.L. contributed equally as first authors.

D.T. and S.E. contributed equally as senior authors.

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What does this study add?

- We show that sebocytes actively contribute to inflammatory processes by recruitment of immune cells into the skin and by skewing T-cell differentiation towards T helper 17 cells.

What is the translational message?

- This interaction of sebocytes might be important in the pathogenesis of other inflammatory diseases.

Sebocytes are specialized epithelial cells that construct the sebaceous gland acini and, together with hair follicles, form the pilosebaceous unit. The main function of sebocytes is considered to be the synthesis and accumulation of lipid droplets, which are released along the hair shaft by cellular disintegration to moisturize the skin surface.[1] More recently, it has become apparent that sebocytes may also act as immunocompetent cells regulating immunological and inflammatory processes in the skin by production of cytokines and inflammatory mediators.[2,3] It was shown that sebocytes constitutively produce CXCL8,[4] and after stimulation with proinflammatory agents, also secrete interleukin (IL)-6.[3] Furthermore, sebaceous triglycerides serve as nutrients for *Propionibacterium acnes*,[5] and their presence stimulates the production of the inflammasome protein IL-1β.[6] In addition, the presence of *P. acnes* may amplify immune responses by stimulating the development of subclasses of T cells.[7,8] It has recently been shown that *P. acnes* induces a T helper (Th)17 response in human peripheral blood mononuclear cells, the expression of key Th17 related genes and IL-17 secretion from $CD4^+$ T cells.[9]

Both sebocytes and *P. acnes* are key players in the pathogenesis of acne vulgaris, which represents a chronic inflammatory disorder of the pilosebaceous unit.[7,10] Various immune factors, including both adaptive and innate immune responses, have been implicated in acne pathogenesis, and the pilosebaceous gland itself also plays an active role in skin inflammation as it releases certain immunological factors such as lipid mediators and proinflammatory cytokines.[11,12] Several studies mapping the progression of inflammatory lesions at different time points revealed that, apart from neutrophils and macrophages, $CD4^+$ cells infiltrate sites of early acne lesions.[13,14]

Although the role of sebocytes in acne inflammation and innate immunity has been widely studied, data regarding the implication of these cells in T-cell recruitment and activation are still missing. Herein, we evaluated whether sebocytes are able to recruit immune cells to sites of skin inflammation and to modulate their function. Moreover, we evaluated a possible synergism between human sebocytes and *P. acnes* in driving an inflammatory response, which may be active in skin homeostasis (symbiosis) and/or in immune reactions such as acne vulgaris.

Materials and methods

SZ95 sebaceous gland cell culture

Immortalized human SZ95 sebocytes[15] were cultured at 37 °C in a humidified atmosphere containing 5% (v/v) CO_2, in Sebomed® medium (Biochrom, Cambridge, U.K.) supplemented with 10% fetal bovine serum (Biowest, Nuaillé, France), 1 mmol L^{-1} $CaCl_2$ solution, 1% penicillin/streptomycin and 5 μg mL^{-1} epidermal growth factor (EGF) (both Sigma-Aldrich, St Louis, MO, U.S.A.). At approximately 80% confluence, SZ95 sebocytes were stimulated with recombinant cytokines (50 ng mL^{-1} each), lipopolysaccharide (LPS)–lipoteichoic acid (LTA) (1 μg mL^{-1}) or *P. acnes* strain 889 (50 : 1 ratio) for 6 h, extensively washed and cultured for additional 24 h in Sebomed medium. SZ95 sebocyte supernatants were collected, filtered using 0·2-μm syringe filters (Sarstedt, Nümbrecht, Germany) and frozen until use in subsequent experiments. Sebomed medium lacking EGF served as a control in stimulation experiments.

Migration assay

Neutrophils, monocytes and T cells were isolated from heparinized whole blood of healthy donors by density centrifugation, CD14 microbeads and CD3 microbeads (Miltenyi Biotech, Bisley, U.K.), respectively, resuspended in complete RPMI 1640 buffer (Invitrogen, Carlsbad, CA, U.S.A.) plus 0·5% bovine serum albumin (Sigma-Aldrich) with a final concentration of 2×10^6 cells mL^{-1} and added to the top of a 5-μm pore polycarbonate membrane (ChemoTx Disposable Chemotaxis System; NeuroProbe, Gaithersburg, MD, U.S.A.). After 2 h at 37 °C with 5% CO_2, migrated cells were further analysed with an LSRFortessa flow cytometer (BD Biosciences, San Jose, CA, U.S.A.). Migrated T cells were additionally stained for CD4, CD8, CD56, CD45RO and CD45RA. Migration was performed in duplicate.

Purification and stimulation of naive and memory $CD4^+$ cells

$CD4^+$ cells were magnetically sorted using the CD4 T Cell Isolation Kit II followed by a positive selection with CD45RO or CD45RA beads (Miltenyi Biotech). A sample of 2×10^5 cells was seeded in a 96-well plate and stimulated with plate-bound human αCD3 and soluble αCD28 (each 0·75 μg mL^{-1}) (BD Biosciences) in the presence of 100 μL SZ95 sebocyte supernatant and 100 μL RPMI 1640 (Invitrogen) supplemented with 2·5% human serum (Lonza, Basel, Switzerland) and 0·5% penicillin–streptomycin solution (Invitrogen) at 37 °C with 5% CO_2. The supernatant of $CD4^+$ $CD45RO^+$ memory T cells was collected at day 3. $CD4^+$ $CD45RA^+$ naive T cells were kept in culture for 6 days, and restimulated for 72 h with plate-bound human αCD3 and soluble αCD28 (each 0·75 μg mL^{-1}) (BD Biosciences) before supernatant collection. Samples were assayed in duplicate.

Dendritic cell generation and mixed leucocyte reaction

$CD14^+$ monocytes (1×10^6) were seeded in a 24-well plate containing 500 μL RPMI 1640 (Invitrogen) supplemented with 1·5% fetal calf serum (Biochrom), 0·5% penicillin–streptomycin solution (Invitrogen), 500 μL of SZ95 sebocyte supernatant and IL-4 and granulocyte–macrophage colony-stimulating factor (100 U mL^{-1} of each) (PromoKine, Heidelberg, Germany) and incubated for 5 days at 37 °C with 5% CO_2. At day 5, dendritic cells (DCs) were stimulated with LPS (1 μg mL^{-1}, Invitrogen) for 24 h, washed twice with phosphate-buffered saline and plated in a 96-well plate in a 1 : 10 ratio with $CD4^+$ $CD45RA^+$ or $CD4^+$ $CD45RO^+$ T cells for the mixed leucocyte reaction. The supernatant of $CD4^+$ $CD45RO^+$ memory T cells was collected at day 3. $CD4^+$ $CD45RA^+$ naive T cells were kept in culture for 6 days and restimulated for 72 h with plate-bound human αCD3 and soluble αCD28 (each 0·75 μg mL^{-1}) (BD Biosciences) before supernatant collection. Samples were assayed in duplicate.

Statistical analysis

Each experiment was performed in technical duplicate. The given n-number represents independent experiments performed with different donors. Statistical analysis was performed using GraphPad Prism software (GraphPad, La Jolla, CA, U.S.A.). Statistical significance between two stimulation conditions was determined using the Wilcoxon matched-pairs signed-rank test. Statistical analysis with more than two stimulation conditions was performed with the Kruskal–Wallis test and Dunn's multiple comparison test to correct for multiple testing. Asterisks represent statistical significance and are defined as $*P < 0{\cdot}05$; $**P < 0{\cdot}01$; $***P < 0{\cdot}001$. If no asterisk is given, no statistical differences could be detected. Graphically, box plots with Tukey whisker plots are shown.

Further information on the culture of immunofluorescence staining, *P. acnes*, cytokine neutralization, protein digestion and enzyme-linked immunosorbent assay/Bio-Plex can be found in Appendix S1 (see Supporting Information).

Results

T helper-17 cells surround the pilosebaceous unit in acne lesions

Staining of paraffin-embedded skin sections of acne lesions revealed a high number of $CD4^+$ $IL\text{-}17^+$ double-positive T cells accumulating in close proximity to the pilosebaceous unit (Fig. 1). Isotype control stainings are given in Figure S1 (see Supporting Information). Despite the fact that healthy control skin did not show signs of inflammation, $CD4^+$ $IL\text{-}17^+$ double-positive T cells were detected next to the sebaceous gland, indicating not only a potential cross-talk of Th17 cells and sebocytes during inflammation, but also homeostasis.

Sebocytes attract immune cells through CXCL8 release

We next analysed whether sebocytes actively contribute to the recruitment of immune cells and especially T cells into the skin. Here we used cell-free supernatants from the human sebaceous gland cell line SZ95, which represents an accepted and widely used model in sebocyte research.[15] In a first step, these supernatants were analysed for production of 27 cytokines, chemokines and growth factors by Luminex technology (Luminex, Austin, TX, U.S.A.), revealing a robust secretion of chemokines such as CXCL8, CCL2, CCL5 and CXCL10 (Table S1; see Supporting Information). As these chemokines are important for granulocyte and leucocyte migration into tissues, we next analysed the migratory capacity of neutrophils, monocytes and T cells towards the SZ95 sebocyte supernatant. Neutrophils, monocytes and T cells migrated towards the SZ95 sebocyte supernatant in a CXCL8-dependent manner (Fig. 2a; Fig. S2a; see Supporting Information). Among T cells, $CD4^+$ and $CD45RO^+$ effector T cells represented the main migratory subsets (Fig. 2b).

Next, we wanted to understand whether cultivation of SZ95 sebocytes in different proinflammatory environments alters the secretion of proteins and also the subsequent migration of cells. Therefore, SZ95 sebocytes were prestimulated with IL-4, interferon (IFN)-γ, tumour necrosis factor (TNF)-α, IL-17, LPS, LTA and *P. acnes*. Whereas all conditions lead to increased secretion of CXCL8, CCL5 and CXCL10, IFN-γ, IL-17, LPS and LTA were the predominant activators of sebocytes (Table S1). Whereas IFN-γ stimulation of sebocytes seems to foster (not significantly) the migration of $CD3^+$ T cells (Fig. 2c), with a significant migration of the $CD45RO^+$ T-cell subset (Fig. S2c), LPS or LTA stimulation significantly induced migration of neutrophils and monocytes (Fig. S2b; see Supporting Information).

These data provide evidence that resting sebocytes can attract immune cells *in vitro* in a CXCL8-dependent manner, and that this chemoattractant effect is further raised in a proinflammatory environment.

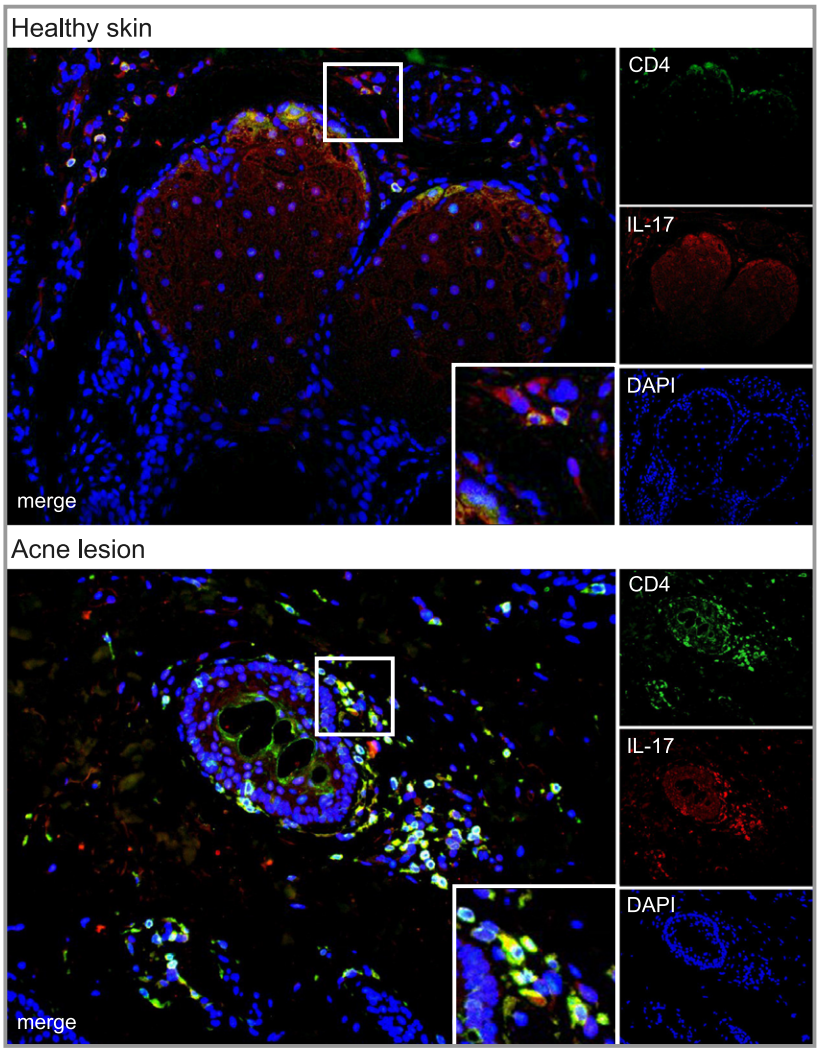

Fig 1. $CD4^+$ interleukin (IL)-17^+ T cells surround the pilosebaceous unit. Paraffin-embedded sections of patients with acne vulgaris were immunofluorescently stained for CD4 (green) and IL-17 (red). The nucleus was counterstained with DAPI (4′,6-diamidino-2-phenylindole; blue). Fluorescence images were obtained using an Olympus IX73 inverted fluorescence microscope equipped with cellSens software (Olympus, Center Valley, PA, U.S.A.) and processed with ImageJ software (https://imagej.nih.gov/ij/). Shown is one representative staining for healthy (upper part) and lesional skin (lower part).

Sebocytes do not influence $CD4^+$ $CD45RO^+$ effector T-cell cytokine secretion

With $CD4^+$ $CD45RO^+$ being the largest T-cell subset that is attracted by sebocytes, we questioned whether their function is actively influenced by sebocytes. Therefore, human, blood-derived $CD4^+$ $CD45RO^+$ cells were stimulated with plate-bound αCD3 and soluble αCD28 in the presence of SZ95 sebocyte supernatant or control medium for 72 h. Here, no significant alteration in the secretion of IL-17, IFN-γ, TNF-α or IL-4 production compared with control medium could be detected. Only for IL-22 secretion was a significant induction detectable (Fig. S3a; see Supporting Information).

As, in this setting, T-cell-receptor stimulation alone might not be sufficient to induce alterations in cytokine secretion, we next investigated whether sebocytes trigger functional changes in T-cell activation via DCs. Therefore, $CD14^+$ monocytes were differentiated into DCs in the presence of SZ95 sebocyte supernatant or control medium and stimulated with LPS prior to coculture with allogeneic $CD4^+$ $CD45RO^+$ cells (mixed leucocyte reaction). SZ95 supernatant did not impact on DC maturation (Fig. S4; see Supporting Information) and also did not impact on T-cell activation, as no significant release of any of the cytokines analysed was detected (Fig. S3b).

Our data suggest that human sebocytes affect memory T-cell cytokine secretion neither directly nor mediated by DCs.

Sebocytes trigger a T helper-17 cell immune response

As naive T cells were also attracted in small numbers by sebocytes, we investigated the influence of sebocytes on T-cell differentiation. For this purpose, $CD4^+$ $CD45RA^+$ T cells were stimulated with αCD3 and αCD28 in the presence of the SZ95 sebocyte supernatant or control medium. As also in our hands intracellular cytokine stainings of differentiated T cells did not work,[16] secretion of effector cytokines was assessed by enzyme-linked immunosorbent assay. Whereas SZ95 sebocyte supernatant did not induce a Th1 or Th2 immune response, as no significant IFN-γ, TNF-α and IL-4 release was detected in the supernatant of differentiated T cells, a significantly higher production of IL-22 and IL-17 was detected after

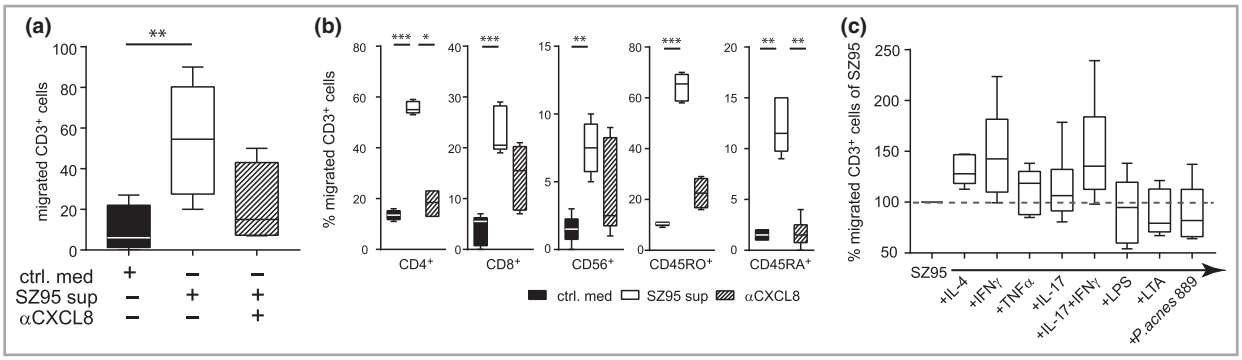

Fig 2. SZ95 sebocytes induce the migration of T cells via secretion of CXCL8. (a) $CD3^+$ T cells migrated towards the SZ95 sebocyte supernatant after 2 h of incubation. Graphs show absolute numbers of migrated cells towards control medium, SZ95 sebocyte supernatant and CXCL8-depleted SZ95 sebocyte supernatant (n = 3). (b) Flow cytometric analysis of $CD3^+$ cells. Migrated $CD4^+$, $CD8^+$, $CD56^+$, $CD45RO^+$ and $CD45RA^+$ cells are expressed as the percentage of total migrated $CD3^+$ cells (n = 3). (c) Flow cytometric analysis of migrated $CD3^+$ cells towards prestimulated SZ95 sebocyte supernatant expressed as the percentage of SZ95 migration. Statistical significance was determined using the Kruskal–Wallis test and Dunn's multiple comparison test to correct for multiple testing, and expressed as $^*P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$; $^{***}P < 0{\cdot}001$. IFN, interferon; LPS, lipopolysaccharide; LPA, lipoteichoic acid; TNF, tumour necrosis factor.

6 days in culture (Fig. 3a). In line with that, DCs that were generated in the presence of SZ95 sebocyte supernatant were also able to drive naive T-cell polarization towards the Th17 phenotype with significantly increased expression of IL-17 and IL-22 (Fig. 3b). Interestingly, IL-17 and IL-22 cytokine levels were even higher compared with the sole stimulation with αCD3 and αCD28, whereas levels of IFN-γ and TNF-α were significantly reduced in this set-up.

Thus, our *in vitro* data imply that human sebocytes have the capacity to skew immune responses towards a Th17 profile.

Sebocytes induce T helper-17 cell differentiation via secretion of interleukin-1β

To address the contribution of lipids or proteins to the observed effect on Th17 differentiation, we incubated the SZ95 sebocyte supernatant with proteinase K. $CD4^+$ $CD45RA^+$ naive T cells were stimulated with αCD3 and αCD28 and cultured in the presence of the protein-digested SZ95 sebocyte supernatant, resulting in a reduction of IL-17 secretion in the absence of the whole-protein fraction (Fig. 4a). This suggests that the sebocyte effect on naive T-cell polarization is primarily protein mediated. However, the effects of sebocytes produced lipids, and interactions of lipids and proteins cannot be ruled out without further experiments.

As, in the steady state, SZ95 sebocytes secrete cytokines that are known to contribute to Th17 polarization, such as IL-1β and IL-6[17] (Table S1; see Supporting Information), we next neutralized these cytokines in SZ95 supernatant and performed a subsequent naive T-cell differentiation. Neutralization of IL-1β led to a decrease of IL-17 secretion in differentiated T cells of 35%, whereas neutralization of TGF-β and IL-6 alone had only marginally effects. Conversely, the depletion of all three cytokines simultaneously abrogated IL-17 production by 44% (Fig. 4b).

Therefore, it is likely that sebocytes drive a Th17 immune response via the production of IL-6, TGF-β and largely IL-1β.

Propionibacterium acnes does not influence immune cell recruitment, but affects the priming capacity of dendritic cells

To explore whether *P. acnes* synergistically acts with sebocytes to reinforce the local symbiosis and/or immune response, SZ95 sebocytes were preincubated for 24 h with *P. acnes* sonicate 889, extensively washed to remove bacteria and further incubated for 24 h prior to supernatant collection. In migration assays, *P. acnes* did not alter the chemoattractant potential of sebocytes compared with SZ95 supernatant, as migration of neutrophils, monocytes or lymphocytes was not altered when sebocytes were preincubated with *P. acnes* (Fig. 2c; Fig. S2b,c; see Supporting Information).

However, when DCs were generated in the presence of *P. acnes*-prestimulated SZ95 supernatant and subsequently used for differentiation of allogeneic $CD4^+$ $CD45RA^+$ naive T cells, these T cells showed a slight, but not significant, increase in IL-17 and IL-22 production (Fig. 5). Interestingly, DCs matured with the SZ95 sebocyte supernatant (either unstimulated or prestimulated with *P. acnes*) induced a significant reduction of the Th1 cytokine IFN-γ ($P = 0{\cdot}03$), whereas TNF-α and IL-4 levels were not significantly altered (Fig. 5).

These data indicate that sebocytes induce Th17 polarization, and *P. acnes* indirectly contributes to this phenomenon by inhibiting Th1 differentiation.

Discussion

Current research on human sebocytes has indicated that these cells are not only bystanders during skin inflammation, but also actively modulate immune responses via secretion of chemokines and cytokines.[2,3,18] In this study, we provide evidence of a functional communication between sebocytes and T cells resulting in the induction of a Th17-dominated immune response. On the other hand, we demonstrate an

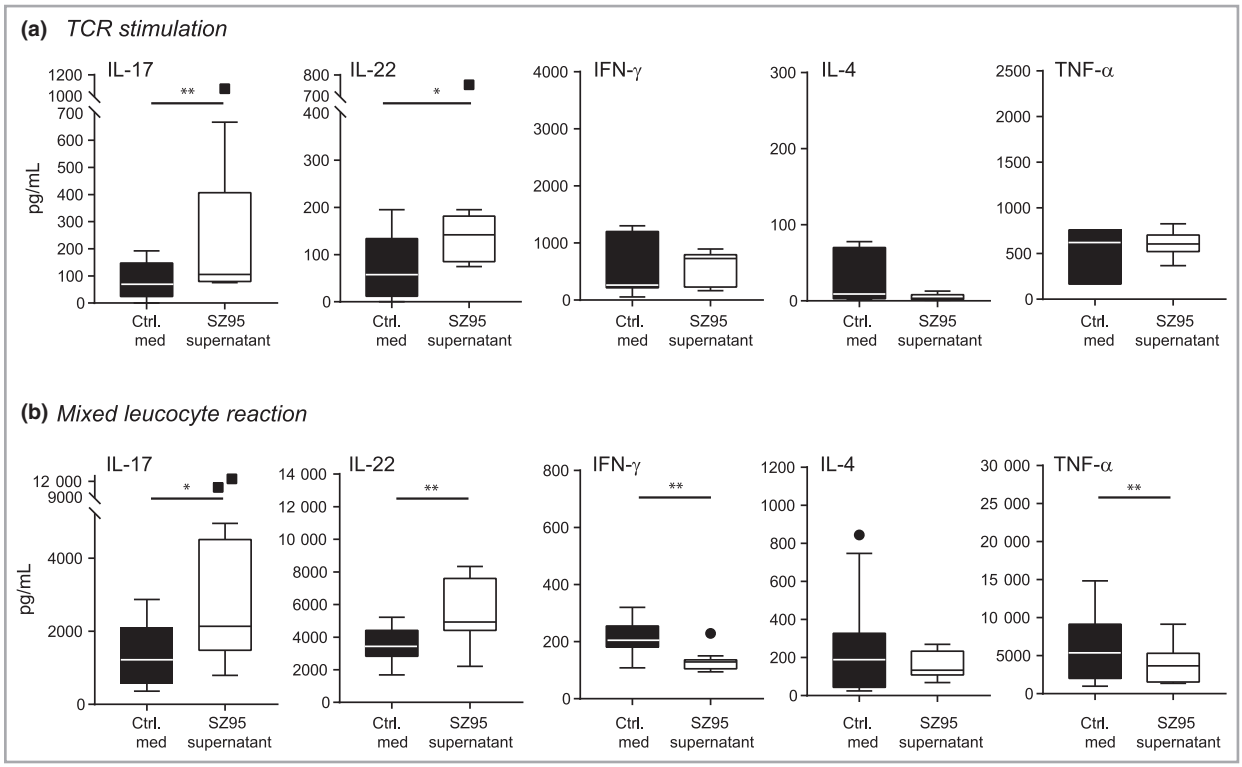

Fig 3. Sebocyte supernatant polarizes naive T cells into T helper-17 cells. (a) $CD4^+$ $CD45RA^+$ naive T cells were cultured with SZ95 sebocyte supernatant or control medium and stimulated with αCD3 and αCD28. After 6 days, cells were restimulated for 72 h and supernatants analysed for levels of interleukin (IL)-17, IL-22, interferon (IFN)-γ, IL-4 and tumour necrosis factor (TNF)-α by enzyme-linked immunosorbent assay (ELISA) (n = 5). TCR, T-cell receptor. (b) Dendritic cells generated from monocytes in the presence of the SZ95 sebocyte supernatant and control medium were stimulated with lipopolysaccharide and cocultured with allogeneic $CD4^+$ $CD45RA^+$ naive T cells. After 6 days, T cells were restimulated with αCD3 and αCD28 for 72 h and supernatants analysed by ELISA (n = 3). Statistical significance was determined using the Wilcoxon matched-pairs signed-rank test and expressed as $^*P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$.

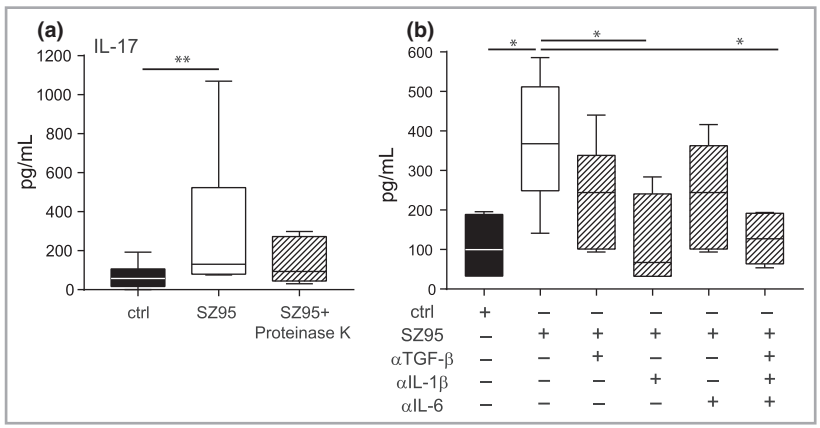

Fig 4. Sebocytes induce T helper-17 cell differentiation by release of key polarizing cytokines. (a) Proteins in SZ95 sebocyte supernatant were removed by digestion with proteinase K. $CD4^+$ $CD45RA^+$ naive T cells were cultured with the protein-depleted supernatant, the entire SZ95 sebocyte supernatant or control medium in the presence of αCD3 and αCD28 antibodies for 6 days. Secretion of interleukin (IL)-17 was measured by enzyme-linked immunosorbent assay (ELISA) after restimulation with αCD3 and αCD28 antibodies (n = 3). (b) SZ95 sebocyte supernatant was incubated for 1 h with transforming growth factor (TGF)-β, IL-1β and IL-6 neutralizing antibodies either alone or in combination and used to differentiate $CD4^+$ $CD45RA^+$ naive T cells for 6 days. After restimulation, levels of IL-17 were measured in the supernatants by ELISA (n = 2). Statistical significance was determined using the Kruskal–Wallis test and Dunn's multiple comparison test to correct for multiple testing and expressed as $^*P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$.

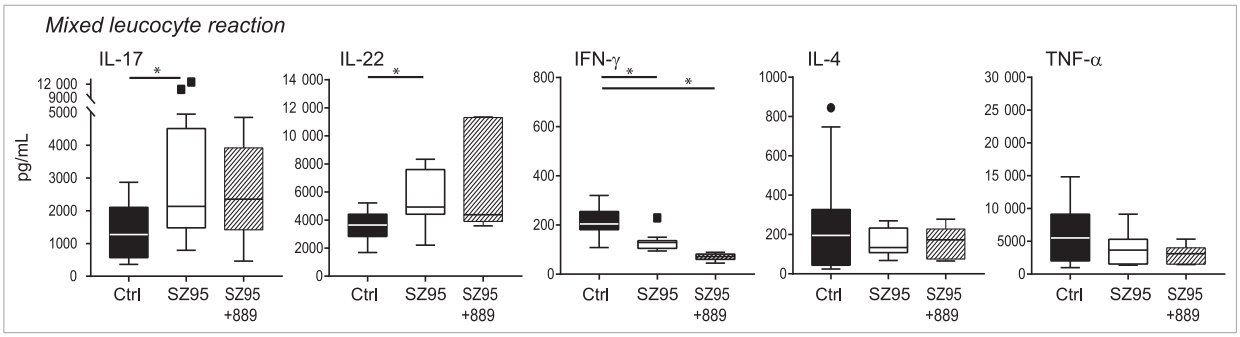

Fig 5. Dendritic cells (DCs) were generated in the presence of interleukin (IL)-4 and granulocyte–macrophage colony-stimulating factor and supernatant derived from SZ95 sebocytes that had been prestimulated with *Propionibacterium acnes* strain 889. At day 5, DCs were stimulated with lipopolysaccharide and subsequently cocultured with $CD4^+$ $CD45RA^+$ naive T cells for 6 days. Differentiated T cells were restimulated with αCD3 and αCD28 antibodies prior to supernatant collection and analysis by enzyme-linked immunosorbent assay for IL-17, IL-22, interferon (IFN)-γ, IL-4 and tumour necrosis factor (TNF)-α levels (n = 3). Statistical significance was determined using the Kruskal–Wallis test and Dunn's multiple comparison test to correct for multiple testing and expressed as $*P < 0{\cdot}05$.

indirect contribution of *P. acnes* via sebocytes and DCs towards acne-associated inflammation.

In the steady state, SZ95 sebocytes release several chemokines and cytokines. This is in line with previously published reports highlighting the *in situ* production of, for example, CXCL8, IL-6 and IL-1β by sebocytes in the sebaceous gland,[3,6] and underlining the importance of SZ95 sebocytes as an *in vitro* model for sebocyte research. Among the steady-state chemokines, CXCL8 has a key role in recruitment of immune cells such as neutrophils and monocytes to sites of skin inflammation.

Although previous reports suggested that neutrophils are the first immune cells in acne lesions,[19,20] some studies revealed that, along with macrophages, T lymphocytes also infiltrate sites of evolving inflammatory lesions.[13,14] Indeed, we found that, in a CXCL8-mediated fashion, sebocytes recruit different subsets of T cells such as $CD4^+$ $CD45RO^+$ effector, but also $CD4^+$ $CD45RA^+$ naive, T cells to the skin. However, during inflammatory responses, sebocytes become further activated by proinflammatory cytokines and/or bacterial products, leading to enhanced secretion of chemokines and cytokines. In line with that, prestimulated SZ95 sebocytes showed an increased chemoattractant potential on immune cells *in vitro* that mainly followed the concentration of CXCL8 and is reflected *in vivo* by high numbers of immune cells surrounding the sebaceous gland in acne lesions.

Owing to the important role of T cells in the inflammatory tissue response, we investigated whether T cells are not only attracted to the sebaceous gland, but also influence their function. We could demonstrate that factors released by sebocytes do not alter cytokine secretion of $CD4^+$ $CD45RO^+$ effector T cells, indicating that sebocytes do not impact on the previously determined T-cell phenotype. One exception is a slight increase in IL-22 production, implying that sebocytes ensure barrier homeostasis by fostering the IL-22/TNF-α axis.[21]

Unlike effector cells, we found that sebocytes impact on the differentiation of $CD4^+$ $CD45RA^+$ naive T cells. Sebocytes secrete various cytokines, most importantly IL-6 and IL-1β, which represent the key cytokines for *de novo* differentiation of Th17 cells.[22,23] Because of this we could show that sebocyte supernatants alone are capable of fully inducing the Th17 phenotype in naive T cells and that this interaction is dependent mainly on IL-1β production. As T-cell priming does not take place in peripheral tissues, we assume that sebocytes contribute to the generation of a local micromilieu that skews differentiation of naive T cells towards the Th17 phenotype in skin-draining lymph nodes.

The Th17 population bridges innate and adaptive immunity and has a key role in mediating host defence. Alone or in synergy, the Th17 effector cytokines IL-17 and IL-22 induce an array of antimicrobial peptides to produce a robust antimicrobial response.[24–26] However, Th17 cells can also induce pathological inflammation and are associated with several inflammatory skin conditions such as psoriasis, atopic eczema and allergic contact dermatitis.[27–29] Moreover, a role for Th17 cells in acne pathogenesis has recently been described. Kelhala *et al.* showed enhanced expression of Th17-associated cytokines and differentiation factors in lesional skin.[30] In line with our data, Agak *et al.* showed that Th17 cells are present in the perifollicular infiltrate of comedones. However, and in contrast to our findings, the authors hypothesized that the Th17 immune response is regulated mainly by *P. acnes*.[9] Similarly, a recent study showed that *P. acnes* induces a Th1/Th17 response even though acne pathogenesis has been associated with Th1-type immunity.[31,32] Our data indicate that sebocytes induce differentiation of neither Th1 nor Th2 cells, but skew the immune response towards a Th17 profile that is further enhanced by the presence of *P. acnes*.

Furthermore, it has been reported that *P. acnes* efficiently induces IL-1β secretion in sebocytes by activating the NLRP3 inflammasome.[6] We could also detect an increase in IL-1β levels when sebocytes were pretreated with *P. acnes*; however, we did not find a further increase in Th17 differentiation, arguing for a quite high intrinsic production of IL-1β that is totally sufficient for Th17 priming, even in a resting state. Our data argue for a steady-state induction of Th17 cells by sebocytes to maintain skin homeostasis. However, when the

pilosebaceous unit is colonized with P. *acnes* under pathological conditions, the Th17 response is further enhanced *in vivo*. In line with this, we could detect $CD4^+$ $IL\text{-}17^+$ cells only sparsely around sebaceous glands in healthy individuals, whereas these cells were frequently colocalized with sebocytes in acne lesions.

Several *in vitro* studies show that P. *acnes* whole cells or cell fractions stimulate cytokine release from immune cells, keratinocytes and sebocytes through binding to Toll-like receptor 2.[2,33–35] However, the mechanism by which P. *acnes* exerts its activity *in vivo* is still unknown. *Propionibacterium acnes* can reside in the deeper portions of sebaceous follicles,[33] but rarely in the sebaceous gland.[36] When this commensal bacterium proliferates, it can come into contact with DCs and activate their maturation, with the consequent immune response crucially depending on the presence of local commensals or pathogens, biofilm production and additional signals from tissue cells.[37]

It has been reported that DCs stimulated with P. *acnes* show an increased expression of adhesive molecules and cytokines, which is similar to DCs activated by LPS.[38,39] In the presence of naive T cells, P. *acnes*-matured DCs induced a strong secretion of IFN-γ that is comparable with LPS-matured DCs, confirming the capacity of P. *acnes* in eliciting a powerful Th1-type immune response.[40] However, the presence of sebocyte supernatant reduced the capability to induce Th1 responses and instead drove the symbiotic and/or immune response to P. *acnes* specifically towards a Th17 commitment. Taken together, we assume that sebocytes maintain the barrier integrity by: (i) homeostatic priming of Th17 cells; (ii) initiation of effective inflammatory responses; and (iii) reduction of pathogenic IFN-γ production to reach homeostasis after inflammation.

The limitations of our study lie clearly in the *in vitro* nature of the performed experiments and the use of a cell line. However, the SZ95 sebaceous gland cell line is a widely accepted and used human sebocyte model that shows comparable behaviour with other sebocyte lines and mostly with primary sebocytes that deliver only limited amounts of material.[41] Furthermore, additional *in vivo* mouse experiments are needed to characterize fully the contribution of sebocyte-induced Th17 cells to acne pathogenesis and, probably, other inflammatory skin diseases.

Despite these limitations, our study provides evidence that sebocytes actively participate in inflammatory processes in the skin via recruitment of immune cells and a functional crosstalk with T cells leading to a pronounced Th17 differentiation. This interaction might be of importance for the pathogenesis of acne vulgaris; however, further studies have to clarify whether the sebocyte–Th17 axis contributes to a beneficial host defence or the perpetuation of a vicious circle of inflammation.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Fig S1. Immunofluorescence isotype controls.

Fig S2. Neutrophils and monocytes migrate towards SZ95 supernatant.

Fig S3. $CD4^+$ $CD45RO^+$ effector T-cell function is not altered by SZ95 supernatant.

Fig S4. SZ95 supernatant does not affect dendritic cell maturation.

Table S1 Bio-Plex results of chemokines and cytokines analyzed in SZ95 supernatant after 24 h of culture.

Appendix S1 Supplementary methods.

Powerpoint S1 Journal Club Slide Set.