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Original Article

Ex vivo culture of lesional psoriasis skin for pharmacological testing

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ABSTRACT

Background: Psoriasis is a chronic, inflammatory skin disorder resulting from a complex interplay between immune and skin cells via release of soluble mediators. While a lot is known about the molecular mechanisms behind psoriasis pathogenesis, there is still a need for preclinical research models that accuratelyreplicate the disease.
Objective: This study aimed to develop and characterize *ex vivo* culture of psoriasis skin as a model for pharmacological testing, where the immunological events of psoriasis can be followed.
Methods: Full thickness punch biopsies of lesional psoriasis skin were cultured in submerged conditions up to 144 h following*in situ* T cell stimulation with rhIL-23 and anti-CD3 and anti-CD28 antibodies. The T cell mediated skin inflammation was assessed by gene and protein l analysis for a panel of inflammatory mediators. Tissue integrity and morphology were evaluated by histological analysis.
Results: T cell stimulation resulted in functional and psoriasis specific*in situ* activation of T cells. The expression levels of most of the proinflammatory mediators related to both immune and skin cells were comparable to these in freshly isolated tissue at 48 and 96 h of culture. Tissue integrity and morphology were sustained up to 96 h. Treatment with a corticosteroid reduced the expression of several pro-inflammatory cytokines and chemokines, whereas anti-IL-17A antibody treatment reduced the expression of the IL-17A downstream markers IL-8 and *DEFB4*.
Conclusion: By preserving keyimmunopathological mechanisms of psoriasis, *ex vivo* culture of psoriasis skin can be used for the investigation of inflammatory processes of psoriasis and for preclinical drug discovery research.

1. Introduction

Psoriasis is a common, chronic T cell mediated skin disease associated with several comorbidities and a low quality of life, affecting 0.09–5.1 % of the population worldwide with significant geographical differences in the prevalence [1]. The IL-23/Th17 immune axis plays a key role in the immunopathogenesis of the disease; inflammatory dendritic cells (DCs) secrete interleukin (IL)-23, which is essential for T helper (Th)17 cell differentiation and sustained IL-17A secreting capacity [2]. Th17 derived cytokines, IL-17A, IL-17A/F and IL-17F are the key drivers of the activation, hyperproliferation and abnormal differentiation of keratinocytes giving rise to the histopathological features of psoriasis including hyperplasia, elongated rete ridges, and para-keratosis. Further, other pro-inflammatory cytokines like tumor necrosis factor (TNF)-α, IL-22 and interferon (IFN)-γ also contribute to the epidermal inflammation [3]. Subsequently, the release of inflammatory mediators like IL-8, and chemokine (C-C motif) ligand-20 (CCL-20) by the activated keratinocytes results in recruitment of additional immune cells [4].

To investigate the immune inflammatory processes and new anti-inflammatory treatments with different mechanisms of action, there is a need for translational preclinical models, which can replicate the immunopathogenic mechanisms of the disease.

Abbreviations: DC, dendritic cell; IL-, interleukin-; Th, cell T helper cell; TNF, tumor necrosis factor; IFN-γ, interferon-γ; CCL-20, chemokine (C-C motif) ligand-20; hBD2, human beta defensin 2; HSE, human skin equivalent; DMSO, dimethyl sulfoxide; ELISA, enzyme linked immunosorbent assay; IHC, immunohistochemical; DAB, diaminobenzidine; Abs, antibodies; K16, keratin 16; BDP, betamethasone dipropionate; HE, Hematoxylin and eosin.

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Xenotransplanted mouse models with human peripheral blood mononuclear cells and/or psoriatic skin grafts using lesional or non-lesional skin are the *in vivo* models bearing closest resemblance to the immunopathogenesis of psoriasis [5,6]. However, the T cell activity has been observed to gradually decline in the grafts during the experimental period [7], and the human immune cells may not be able to establish cross talk with the murine host cells given the cross species differences [8]. As an alternative to animal models, different skin explant models with healthy human skin and human skin equivalent (HSE) models have been developed for psoriasis [9]. The major challenges of these models are associated with incorporation of immune cells of specific phenotype of relevance in psoriasis, as well as with replicating the histopathological features of psoriasis [9]. Short term (12−14 hrs) *ex vivo* culture of psoriasis skin for test of anti-inflammatory compounds has previously been reported by others, but a characterization of the psoriasis phenotype upon *ex vivo* culture has not been included [10,11].

To overcome the limitations of the above mentioned models, we have also previously employed *ex vivo* culture of psoriatic keratome biopsies [12]. Briefly, the key cellular activities and inflammatory features as found in psoriatic skin *in vivo* were resumed in the keratome biopsies by *in situ* activation of T cells and maintained for up to 72 h. However, limitations of this model were short culture time due to rapid loss of tissue integrity and limited amount of dermis. Based on learnings from this model, we further developed an *ex vivo* culture of psoriasis skin using full-thickness biopsies, where the complex immunopathological events of psoriasis are even better maintained, and which can be modulated by pharmacological intervention.

2. Materials and methods

2.1. Human specimens

Full thickness punch biopsies, 5 mm of diameter, were obtained from active lesions of untreated patients with psoriasis vulgaris after approval from local ethical committees and the Danish Data Protection Agency and upon written informed consent by the patients. For each patient, a maximum of 6–7 adjacent biopsies were taken from the center of the same active lesion. The biopsies were either immediately snap frozen or fixed in formalin for protein and histological analysis, respectively, or were transported in cold Dulbecco's modified Eagle medium containing 100 μg/ml gentamicin, 100 U/ml penicillin, 100 μg/ml streptomycin and 2.5 μg/ml Fungizone (All from Gibco, Life Technologies, Carlsbad, CA, USA) up to arrival in the laboratory.

2.2. Psoriasis skin explant culture

The biopsies were placed in culture within maximum 30 h after removal from the patient. The punch biopsies were cut in half and each half served as an individual sample. The tissue was cultured in 24-well plates in 800 μl of EpiLife medium with 10 ng/ml rhIL-2 (Gibco, Life Technologies, Carlsbad, CA, USA), growth supplements and antibiotics. For *in situ* activation of T cells, 1 μg/ml anti-CD3 (Cat:MAB100) and anti-CD28 (Cat: MAB342) antibodies or Mouse IgG1 Isotype Control (Cat: MAB002), and 50 ng/ml rhIL-23 (Cat:1290-IL) (all from R&D Systems, Minneapolis, MN, USA) were used. The biopsies were cultured either for 2, 5, 15, 24, 48, 96 or 144 h. Following *ex vivo* culture, the tissue samples and culture supernatants were harvested and stored at −80 °C or fixed in 4 % formaldehyde until analysis. A schematic representation of the study design (Figure S1) and a more detailed description of culture conditions are included in the supplementary materials.

2.3. Test compounds

For treatment with corticosteroid, the samples were treated at the start of the culture with either 1 μM betamethasone dipropionate (BDP), or matching vehicle control (0.1 % dimethyl sulfoxide) for 48 h or 96 h. Stock solution (10 mM) of BDP (provided by LEO Pharma A/S) was prepared in dimethyl sulfoxide (DMSO) (Sigma–Aldrich, St. Louis, MO, USA). The stock solution was diluted in tissue culture medium at the time of use to the final concentration of 1 μM. The final concentration used in this study is based on exposure data in human skin after topical application of calcipotriol/betamethasone fixed-combination product, as previously reported [12]. For treatment with antibody, the samples were treated at the start of culture with either anti-IL-17A antibody, or an isotype control, for 48 or 96 h. Anti-human IL-17A antibody (Cat: AF-317-NA, R&D Systems Minneapolis, MN, USA) was diluted in culture medium at the time of use to a final concentration of 40 μg/ml. The final concentration used in this study is based on the neutralization dose (ND_{50}) of the neutralising antibody reported by the manufacturer and chosen to be ~300-fold higher than the expected levels of IL-17A in the skin samples to ensure a complete neutralization of IL-17A. For isotype control a polyclonal goat IgG (Cat: AB-108-C, R&D Systems, Minneapolis, MN, USA) was used.

2.4. Histology

The biopsies were fixed in 4 % formaldehyde for 24−48 h at room temperature prior paraffin embedding. Sections (4 μm) were stained with hematoxylin and eosin (HE) for analysis of tissue integrity.

2.4.1. Immunohistochemistry (IHC)

Sections (3 μm) were used for IHC staining. IHC was performed following standard staining methods with minor variations on an automated Leica Bond platform (BOND RX Fully Automated Research Stainer, Leica Biosystems Nussloch GmbH, Germany). Stimulating antibodies (anti-human CD3 and CD28 antibodies) were detected with BOND Polymer Refine Detection (Cat: DS9800, Leica Biosystems, Nussloch, Germany), which includes post primary rabbit anti-mouse IgG antibody. K16 was detected with rabbit anti-K16 antibody (0.5 μg/ml) (Cat no.:Ab76416, AbCam, Cambridge, United Kingdom) and CD3 with rabbit anti-CD3 antibody (3 μg/ml) (Cat no.:A0452, Agilent, California, United States). All the stains were performed with appropriate positive and negative controls and isotype controls. A more detailed description is included in the supplementary materials.

2.5. Protein analysis

Protein analysis was performed on both culture supernatants and tissue lysates. The method for tissue lysis is described in supplementary materials. The protein levels of IL-22 and IL-17F were measured with ELISA, using Human IL-22 Quantikine ELISA kit and Human IL-17F DuoSet ELISA Kit (both from R&D Systems). The MSD platform (Meso Scale Discovery, Rockville, MD, USA) was used for measuring IL-17A/F (U-PLEX biomarker Group 1 (hu) Individual assay IL-17A/F), IL-4, IL-5, IL-13, TARC, IL-17A, IFN-γ, TNF-α, CCL-2 and IL-1α (Human U-PLEX), IL-8 (Human IL-8 (HA) V-PLEX) and CCL-20 (Human Cytokine assay: Tissue culture kit, CCL-20). All analyses were preformed according to the manufacturer's instructions. Protein levels were quantified according to a standard curve prepared using a similar skin matrix and expressed in pg/ml. Protein levels from tissue lysates were adjusted for the protein recovery efficiency.

2.6. Quantitative real-time RT-PCR (qRT-PCR)

Total RNA was extracted by using the mirVana kit (Life Technologies) according to the instructions provided by the manufacturer and Precellys® 24 tissue homogenizer (Bertin instruments, Montigny-le-Bretonneux, France) for the mechanical lysing of the tissue. cDNA synthesis was performed with the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). cDNA was amplified by quantitative real-time PCR using Taqman® Gene Expression Assays. (Assay IDs are listed in the supplementary materials.)

2.7. Statistical analysis

Means and SEM are calculated from the repeated experiments. Values are represented as relative gene expression, protein

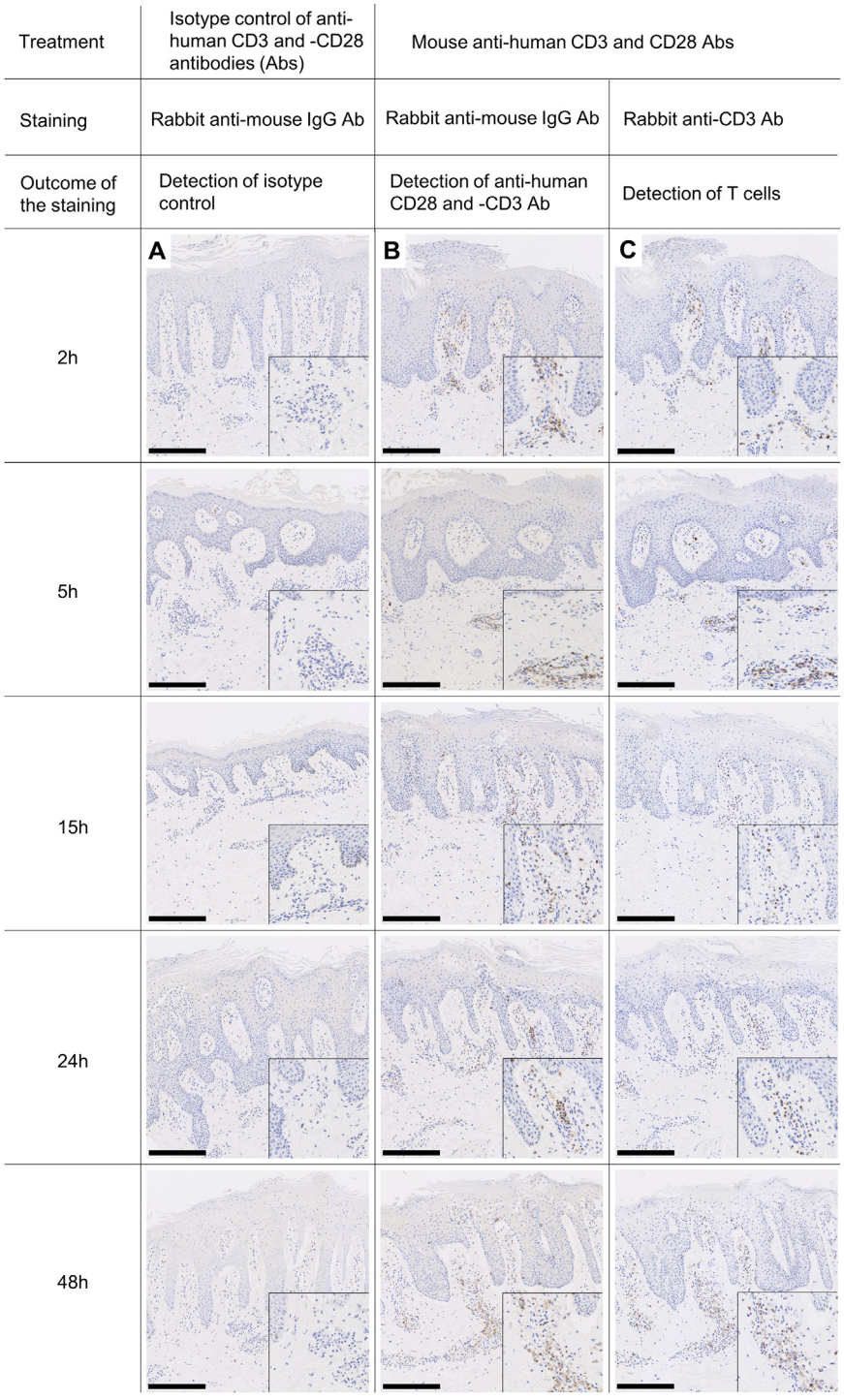

Fig. 1. Co-localization of mouse anti-human CD3 and CD28 antibodies and T cells. Full thickness punch biopsies were cultured in submerged conditions for 2, 5, 15, 24 or 48 h with addition of rhIL-23 and anti-CD3 and anti-CD28 or isotype control antibodies. The tissue was formalin fixed and paraffin embedded at each indicated culture time point. A) IHC staining of isotype control treated samples targeting mouse IgG for detection of the isotype control. B) IHC staining of anti-CD3 and-CD28 antibody treated samples targeting mouse IgG for detection of anti-CD3 and anti-CD28 antibodies. C) IHC staining of T cell stimulated samples targeting human CD3 for detection of T cells. The bars equal to 250 μm. One representative donor out of 4 is shown (n = 4).

concentrations in pg/ml or in the case of pharmacological studies as percentage of the vehicle control (100 %) for each experiment. By the use of GraphPad Prism software, statistical analysis was performed with Wilcoxon signed-rank test (*P ≤ 0.05; **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001).

3. Results

3.1. In situ *stimulation resumes T cell activity in* ex vivo *culture of psoriatic skin*

In the *ex vivo* culture of psoriatic keratome skin, T cell activity is resumed by *in situ* T cell activation using anti-CD3 and anti-CD28 antibodies [12]. Therefore, we investigated, whether *in situ* activation of the T cells in psoriatic full thickness punch biopsies is feasible and necessary.

Firstly, the penetration and distribution of the T cell-activating antibodies were addressed by culturing the biopsies in the presence of rhIL-2 alone or with rhIL-2, rhIL-23, and mouse anti-human CD3 and anti-human CD28 antibodies, or isotype control (mouse IgG) for 2, 5, 15, 24 or 48 h. IHC analysis of tissue stained with rabbit anti-mouse IgG clearly shows the presence of mouse anti-human CD3 and CD28 antibody (Fig. 1, B) but not of mouse isotype control antibody (Fig. 1, A) indicating that only T cell stimulating antibodies (anti-human CD3 and CD28) were able to distribute within the skin due to their high affinity to the targets. The presence of mouse anti-human CD3 and CD28 was observed in the samples already after the first 2 h of culture (Fig. 1, B). Furthermore, the T cell stimulating antibodies were found to co-localize with the T cell infiltrates in the tissue, as demonstrated by the similar expression pattern of CD3 (Fig. 1, C).

Secondly, we investigated whether *in situ* T cell stimulation results in functional T cell activation by following their capacity to secrete proinflammatory cytokines. It was found that the level of IL-17A released in the culture media was already increased after 15 h of culture, and being most prominent after 48 h compared to both unstimulated and isotype treated controls (Fig. 2). Similar results were obtained for IL-22, IL-17F, IL-17A/F and IFN-γ (Supplementary, Figure S2). Undetectable or low levels of the Th2 related cytokines IL-13, IL-5 and IL-4 and the chemokine TARC were found in the tissue culture supernatants during the entire culture period (Supplementary, Figure S3), indicating that the psoriasis associated Th17 and Th1 phenotypes were maintained during the culture.

Taken together, the *in situ* T cell stimulation with anti-CD3 and anti-CD28 antibodies results in functional T cell activation and is necessary for creating a window of expression, as demonstrated by approximately 4-fold increase of IL-17A expression at 48 h of culture in the stimulated samples, when compared to the isotype control.

3.2. *Maintenance of the psoriatic inflammatory phenotype and histopathological features in prolonged culture*

We confirmed that T cells can be activated by the exogenous *in situ* activation and that the phenotype of the Th17 cells is maintained for at least 48 h. Consequently, we further investigated, whether the culture period can be prolonged beyond 48 h, and whether the inflammatory profile of the model is comparable to that of psoriasis lesions *in vivo*.

The tissue morphology and integrity was evaluated by HE staining (Fig. 3, A). As an effect of the submerged culture conditions, spongiosis of the tissue was detectable after 48 h of culture manifested as swelling of the cells in the epidermis. However, the overall tissue integrity was acceptable and maintained up to 96 h. At 144 h of culture, the tissue integrity was clearly decreased, which was seen as pronounced detachment of the epidermis from the dermis in some of the samples as well as necrotic areas in the epidermis (Fig. 3, A, panel far right, black arrow). However, the classical psoriatic features, such as epidermal hyperplasia, parakeratosis and the elongation of rete ridges, were maintained throughout the culture time. T cells were maintained in the tissue at all the measured time points, as shown by the CD3 IHC staining (Fig. 3, B). Additionally, it was found that only a

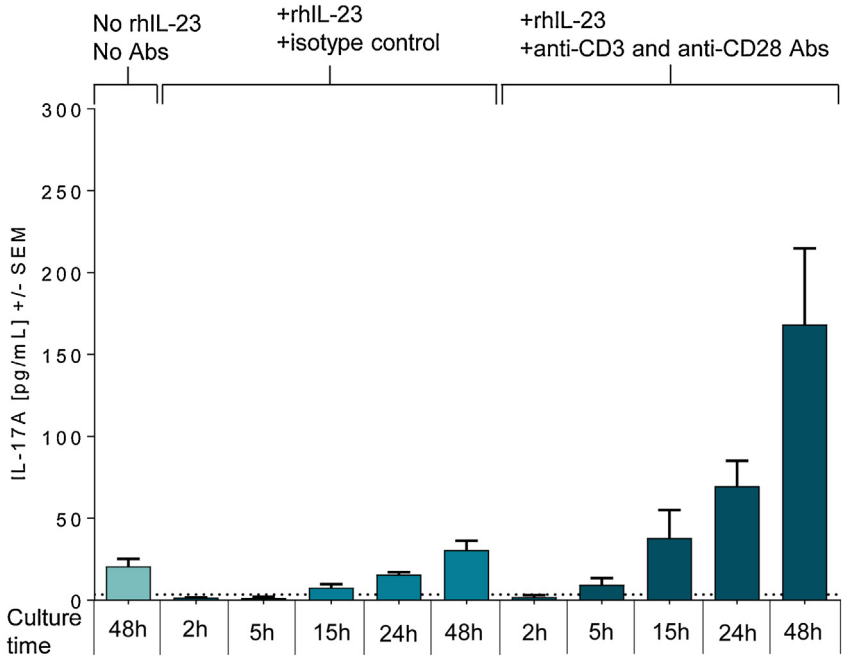

Fig. 2. IL-17A expression is increased by T cell stimulation after 15 h, 24 h and 48 h of culture. The psoriatic full thickness punch biopsies were cultured for 2, 5, 15, 24 or 48 h with or without rhIL-23 and anti-CD28 and anti-CD3 antibodies or with rhIL-23 and isotype control. The IL-17A protein levels were measured in the tissue culture supernatants using MSD platform. The data was obtained for 4 different donors in one experiment (n = 4). The dotted line indicates LLOQ. The error bars express ± SEM.

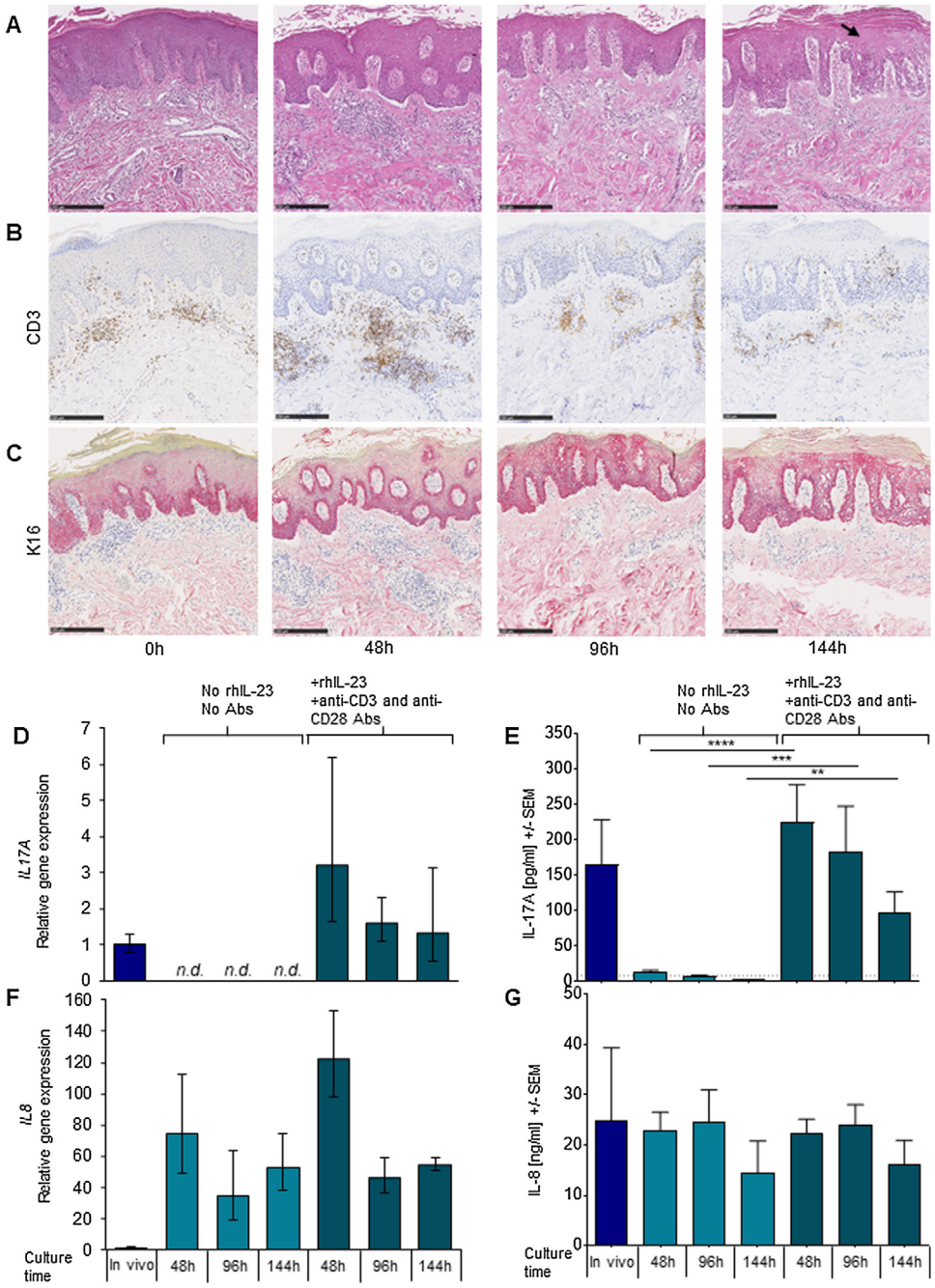

Fig. 3. The tissue integrity and the psoriasis phenotype is maintained up to 96 h of culture. For the histological analysis full thickness punch biopsies were either formalin fixed immediately after arrival to the laboratory (denoted as 0 h) or cultured in submerged conditions for 48 h, 96 h or 144 h with addition of rhIL-23 and anti-CD3 and anti-CD28 antibodies. The tissue was formalin fixed and paraffin embedded in the end the culturing period. A) HE staining, B) IHC staining for CD3 C) IHC staining for keratin 16. The bars equal to 250 μm. The results of one representative donor out of six is shown (n = 6). For the mRNA and protein analysis, 6 or 7 psoriatic full thickness punch biopsies were obtained per donor. 1 biopsy was snap frozen immediately after isolation from the donor and the rest were cultured for 48, 96 or 144 h with or without rhIL-23 and anti-CD28 and anti-CD3 antibody stimulation. The mRNA expression was measured in tissue lysates of the cultured and the snap frozen biopsies. D) IL17A and F) IL8 mRNA expression was normalized to GAPDH, PGK1 and PPIA reference genes. The relative gene expression values ± upper and lower limits are mean values from independent experiments using skin samples from three different donors (n = 3). Protein levels of E) IL-17A and G) IL-8 were measured in tissue culture supernatants and in tissue lysate of snap frozen biopsies (samples denoted as 0 h) using MSD platform. The samples were obtained from six different donors (n = 6) from two independent experiments. The dotted line indicates LLOQ. The error bars express ± SEM. Wilcoxon signed-rank test was used to compare the mRNA and protein expression levels (****$P \leq 0.0001$; ***$P \leq 0.001$; **$P \leq 0.01$).

minimal number of T cells had migrated out of the tissue (data not shown). Keratin 16 (K16) expression, a marker for keratinocyte hyperproliferation, remained stable throughout the culture period, as shown by the K16 IHC staining (Fig. 3, C). T cell stimulation did not affect the tissue integrity, retainment of T cells in the tissue or the expression of keratin 16. Finally, protein levels of IL-1α released in the tissue culture supernatant were assessed to address signs of cellular toxicity and irritation. The levels were found to be stable throughout the culture time, and not affected by the T cell stimulation (Supplementary, Figure S4).

To evaluate the inflammatory profile of the cultured skin, IL-17A expression was measured in samples cultured with rhIL-2 and either with or without the addition of rhIL-23, anti-CD3 and anti-CD28 antibodies for 48, 96 and 144 h, and in tissue lysates prepared

from biopsies, which were snap frozen immediately after removal from the patient. For the cultured samples the IL-17A protein expression was measured in the tissue culture supernatant and the mRNA expression in the tissue. The *in situ* T cell stimulation increased the expression of IL-17A at all the measured timepoints both at the mRNA level (Fig. 3, D) and protein level (Fig. 3, E) compared to unstimulated control, but the expression declined during the culture time. Importantly, the secreted levels of IL-17A in the supernatants were comparable to the levels detected *in vivo*. Similarly, *in situ* T cell stimulation also increased the expression of IL-17F, IL-17A/F, IL-22, and TNF-α compared to unstimulated control (Supplementary, Figure S5). However, the protein levels of IL-17F and IL-17A/F were approximately 5-fold higher in the *ex vivo* culture setting compared to the levels detected *in vivo* (Supplementary, Figure S5). Compared to the *in vivo* samples, the levels of IL-22 secreted in the supernatant were initially higher after 48 h of *ex vivo* culture, but similar after 96 and 144 h of culture (Supplementary, Figure S5). The secreted level of TNF-α in the culture media were approximately 6-fold lower compared to the *in vivo* samples (Supplementary, Figure S5).

To examine whether the expression of keratinocyte-derived chemokines is maintained during the culture period, and whether the expression resembles the levels found *in vivo* in psoriasis, the level of the IL-17A-induced chemokine, IL-8, was measured. Compared to *in vivo* samples, the *ex vivo* T cell stimulation resulted in an increased mRNA expression, whereas at the protein level IL-8 was found to be expressed in the same range in the tissue culture supernatant as *in vivo* (Fig. 3, F and G). Further, the expression of IL-8 was high and not further increased by the T cell stimulation at any time point neither at protein level (Fig. 3, F) or mRNA level (Fig. 3, G).

The induction of IL-17A, IL-17F, IL-17A/F, IL-22 and IFN-γ protein expression was investigated also without the exogenous addition of rhIL-23. The expression of these cytokines could be induced by stimulation with anti-CD3 and anti-CD28 antibodies alone, but the addition of rhIL-23 further increased the expression. The keratinocyte-derived chemokines CCL-20, CCL-2 and IL-8 were not affected by the addition of rhIL-23 (Supplementary, Figure S6).

3.3. Translational and pharmacological evaluation of the model

Having established that the cultured tissue replicates the key molecular and histopathological features of psoriasis inflammation and that the psoriasis phenotype can be sustained up to 96 h, the feasibility of using this model for pharmacological studies was also investigated.

Two types of treatment with different mechanisms of action were tested. Betamethasone dipropionate (BDP), representing a corticosteroid treatment, and a commercially available anti-IL-17A antibody, representative of a biological treatment used in the clinic, were applied in the beginning and throughout the culture period. BDP decreased the protein levels of IL-17A (Fig. 4, A) and mRNA levels of *IL17A* and *DEFB4* (Fig. 4, B and C) after 96 h of culture, compared to the vehicle control. Similar results were found for IL-8, CCL-2, IFN-γ, TNF-α and IL-22 (Supplementary, Figure S7). The antibody treatment significantly inhibited the protein expression of IL-8 (Fig. 4, D) and the mRNA expression of both *IL8* and *DEFB4* after 96 h of culture, when compared to isotype control (Fig. 4, E and F). Similar results were found for IL-17A, *IL23A* and *CXCL1* mRNA expression (Supplementary, Figure S8). It has been previously shown that psoriatic keratinocytes express IL-23 [13,14]. Our results suggest that the downregulation of IL-23 expression in anti-IL-17A antibody treated samples could be due to either direct or secondary effects by blocking IL-17 signalling in keratinocytes. The level of IL-22 were not affected by anti-IL-17A antibody treatment (Supplementary, Figure S8).

4. Discussion

In order to understand how immunomodulatory drugs affect the interplay between different cell types, and may improve psoriasis, there is a need for translational models, where the immunological events of psoriasis are well replicated. In our model, full thickness punch biopsies of lesional psoriatic skin were cultured under submerged conditions. Previously Lovato et al. [12] described that T cells within keratome skin samples from psoriasis patients need to be activated *ex vivo* in order to regain the inflammatory activity as *in vivo*, and therefore rhIL-2, rhIL-23, anti-CD3 and anti-CD28 antibodies were added to the culture medium. In line with these findings, we showed that *in situ* stimulation of skin T cells by the same stimulation cocktail as in the keratome cultures resulted in a psoriasis-specific expression of proinflammatory cytokines maintained for up to 96 h of culture and at levels comparable to the expression observed *in vivo* for most of the cytokines. Also, the histopathological features of psoriasis were retained and tissue integrity remained acceptable up to 96 h of culture, altogether suggesting that the psoriasis inflammation and psoriasis-like tissue morphology are maintained under the culture conditions. The presence of these features is advantageous, since they are rarely adequately achieved in *in vitro* models, such as reconstructed skin models (reviewed in [9]).

Stimulation of T cells without addition of exogenous IL-23 resulted in an upregulation of several T cell-derived cytokines, indicating that the model can be modified to specific needs, such as for investigating the effect of treatments targeting the IL-23/IL-12 pathway. In fact, mRNA expression of IL-23 was found to be on par with levels detected *in vivo* throughout the culture time (Supplementary, Figure S9). However, as the biggest window for investigating treatment effect on T cell activity is reached by addition of IL-23, this approach is preferable for pharmacological studies, whenever compatible.

We demonstrated the feasibility and relevance of the psoriasis *ex vivo* culture as a model for pharmacological studies by testing the effect of two treatments, with clinically validated targets, on the expression of the key markers of psoriasis. The corticosteroid treatment resulted in a significant decrease in both T cell-and keratinocyte-derived soluble mediators, while the treatment with an anti-IL-17A antibody resulted in an immunological modulation of the cross-talk between T cells and keratinocytes with significant inhibition of the expression of IL-8 and *DEFB4*. Of note, it is clear from the data that the responses to *in situ* T cell activation and to the two types of treatment are donor dependent. However, it is important to keep in mind that the variation in the data reflects what is also seen in clinical studies. As an example, the anti-IL-17A antibody secukinumab downregulates the protein expression of human beta defensin (hBD)-2 in psoriasis patients, but the expression levels among individual patients vary broadly both before and after treatment [15].

The keratinocyte-derived proinflammatory mediators were not found to be affected by the T cell stimulation to the same extent and were readily produced in the culture, suggesting that there is a difference between keratinocytes and T cells in their activation state. Reasons for this phenomenon are yet to be elucidated, but the stimulation-independent expression of proinflammatory mediators could be a response to the mechanical trauma induced, when the biopsy is taken, or a response to the culture conditions, or a combination of both. However, the keratinocyte responses were T cell-dependent, since it was possible to downregulate the expression of the keratinocyte-derived IL-8 significantly by anti-IL-17A treatment.

The tissue integrity was acceptable at 96 h, but clearly compromised at 144 h of culture. The histological observations are supported by the finding that the expression of T cell-and

keratinocyte-derived soluble mediators declined at 144 h of culture. These results lead us to conclude that the reliability of the model is sustained up to 96 h, whereas any results obtained on a later time point should be critically reviewed. A culture time of 96 h is significantly shorter than what was reported previously by Varani et al. [16] in a similar experimental set up, where psoriatic full thickness biopsies were cultured for 8 days in submerged conditions, but without exogenous T cell stimulation and in KBM culture medium supplemented with 1.4 mM Ca^{2+}. However, in that study the characterization of the psoriasis phenotype was limited to evaluation of the histological features of the tissue at day 0 and 8. Others have also reported the use of *ex vivo* culture of psoriasis skin for testing effects of anti-inflammatory compounds [10,11]. However, in these studies characterization of the psoriasis

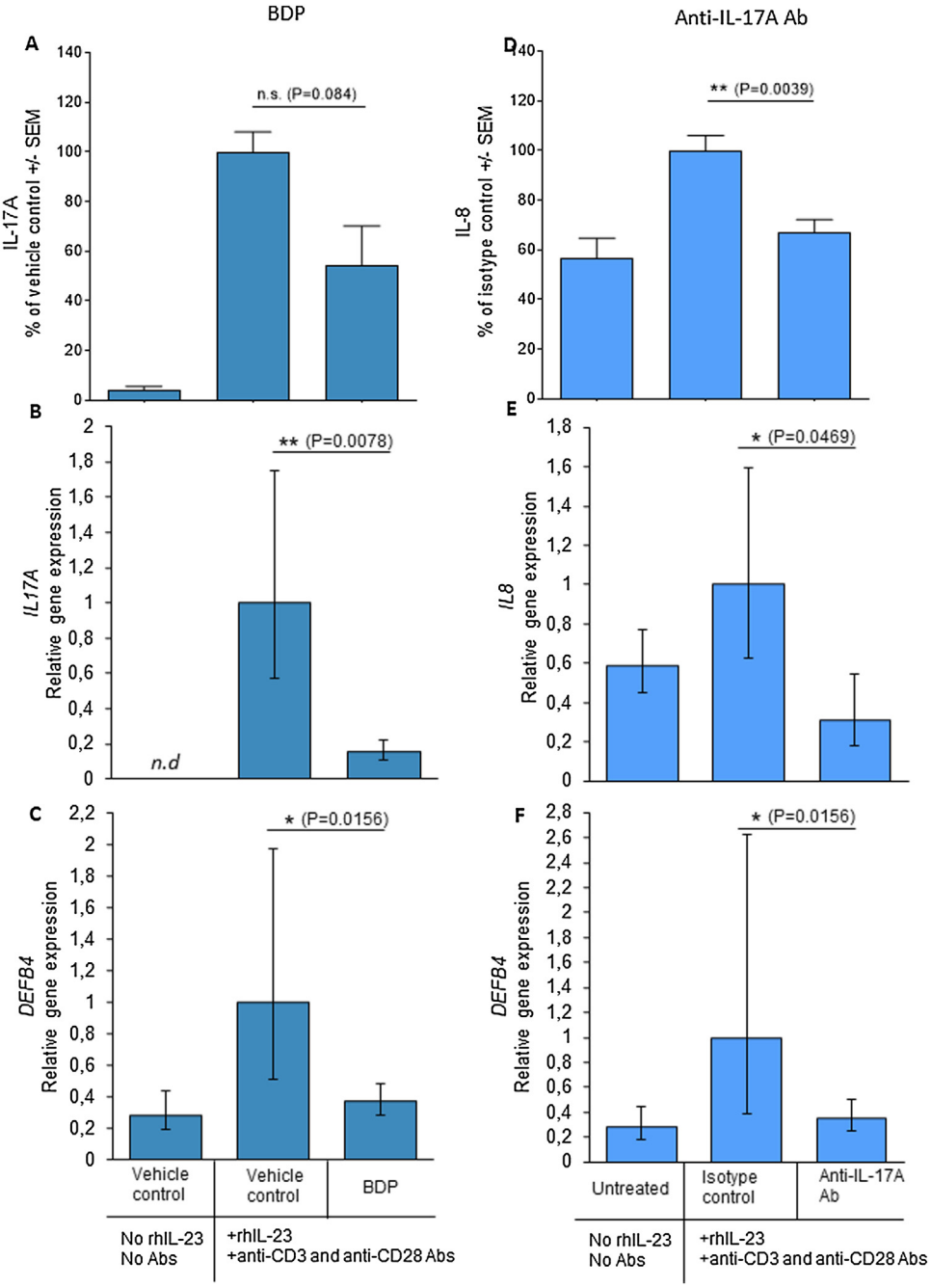

Fig. 4. Treatment effect by betamethasone dipropionate and anti-IL-17A antibody. Full thickness punch biopsies were cultured in submerged conditions for 96 h either with or without addition of rhIL-23 and anti-CD3 and anti-CD28 antibodies. Betamethasone dipropionate BDP (1μM) or vehicle control (0.1 % DMSO) or anti-IL-17A antibody or corresponding isotype control were added to the culture medium in the beginning of the culture period. The levels of soluble mediators were measured in the tissue culture supernatant using MSD platform and the mRNA expression was measured in tissue lysates of the cultured biopsies. A) IL-17A protein expression upon treatment with BDP, B) IL17A mRNA expression upon treatment with BDP, C) DEFB4 mRNA expression upon treatment with BDP, D) IL-8 protein expression upon treatment with anti-IL-17A antibody, E) IL8 mRNA expression upon treatment with anti-IL-17A antibody, F) DEFB4 mRNA expression upon treatment with anti-IL-17A antibody. The protein expression expressed as the percentage difference compared to vehicle treatment control are the mean values ± SEM measured in five different donors (n = 5) from three independent experiments. The mRNA expression of IL8 and DEFB4 was normalized to GAPDH, PGK1 and PPIA. The relative gene expression values ± upper and lower limits are mean values from three independent experiments from four different donors (n = 4). Wilcoxon signed-rank test was used to treatment effect (****$P \leq 0.0001$; ***$P \leq 0.001$; **$P \leq 0.01$; *$P \leq 0.05$).

phenotype upon *ex vivo* culture was not described and the culture time was shorter compared to our model, limiting the feasibility to investigate effects by anti-inflammatory compounds [10,11]. On the contrary, we demonstrated that the time frame in our model is sufficient to see treatment effects of both a small molecule and an antibody.

It is important to stress that the effects of submerged culture conditions are not entirely known, and it can be assumed that it affects the tissue integrity of the psoriasis skin negatively. Indeed, several studies have been made to characterize healthy human skin explants in culture, including commercially available models like NativeSkin® from Genoskin [17], where skin explants are cultured at air-liquid interface and the tissue integrity is maintained for a prolonged period of time [18–20]. A little light is shed on this matter in the study by Peramo and Marcelo, 2012 [18], where full thickness biopsies of healthy human skin were cultured either in a transwell system at air-liquid interface or semi-submerged in the culture medium. They found that the tissue integrity of biopsies cultured in submerged conditions declined faster compared to the tissue cultured at air-liquid interface. Interestingly, Companjen et al. [21] state that healthy skin deteriorates slower compared to psoriatic skin, when cultured at air-liquid interface, but the deterioration can be largely avoided by culturing the psoriatic skin in an atmosphere containing 95 % oxygen. These studies indicate that the model could potentially be further optimized by air exposure, if a suitable technical setting can be found for relatively small biopsies.

In conclusion, we present here a well-characterized model using *ex vivo* culture of psoriasis full thickness biopsies. Although one limitation of our model is the lack of feasibility to test topical drugs as the skin samples are submerged in the culture media, we provide a thorough characterization of its feasibility to maintain psoriasis inflammatory responses and to be employed for pharmacological studies as well as for elucidating the immuno-pathological events of psoriasis.

Declaration of Competing Interest

The authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jdermsci.2019.12.010.

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