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Impaired Langerhans' cell migration in psoriasis is due to an altered keratinocyte phenotype induced by interleukin-17

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Running title:

Impaired LC migration in psoriasis is a result of altered keratinocyte phenotype.

Key words

Psoriasis, Langerhans cell, keratinocytes, interleukin-17.

Abbreviations:

BSA; bovine serum albumin, ELISA; enzyme linked immunosorbant assay, FCS; foetal calf serum, GAPDH; glyceraldehydes 3-phosphate dehydrogenase, IL; interleukin, KC; keratinocyte LC; Langerhans cell, MMP; matrix metalloproteinase, PBS; phosphate buffered saline, PN; uninvolved non-lesional psoriasis skin.

WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?

- Langerhans' cells (LC) are the dendritic cells of the epidermis and regulate the cutaneous immune response.
- LC migration is impaired in the uninvolved skin of patients with early-onset chronic plaque psoriasis.
- Interleukin (IL)-17 is a cytokine that is involved in the pathogenesis of psoriasis.
- We have recently shown that IL-17 inhibits LC migration in a healthy human *ex vivo* epidermal explant model.

WHAT DOES THIS STUDY ADD?

- Conditioned media generated from psoriasis, but not healthy, keratinocytes (KC), inhibits LC migration.
- Conditioned media generated from healthy KC treated with IL-17 also inhibits LC migration.
- This suggests that KC, in response to increased expression of IL-17, are responsible for secreting a factor that alters LC function.
- In an *ex vivo* psoriasis explant model addition of neutralising anti-IL-17 antibody restores LC migration.

ABSTRACT

Psoriasis is a common skin condition driven by increased expression of interleukin (IL)-17. Langerhans' cells (LC) are epidermal dendritic cells that regulate cutaneous immune responses. Within uninvolved skin of patients with psoriasis, LC display impaired migration from the epidermis. Here the role of keratinocytes (KC) in the regulation of LC function, and the response of KC to IL-17 has been investigated. Keratinocytes were cultured from the uninvolved skin of psoriasis patients and healthy individuals with or without IL-17 treatment and the conditioned medium examined for its ability to alter LC function in an *ex vivo* human skin explant model. Furthermore, we examined the effect of IL-17 on LC mobilisation in psoriasis by neutralising IL-17 in the same skin explant model. We found that conditioned medium from psoriasis KC inhibited LC migration in healthy skin. Moreover, conditioned medium from healthy KC treated with IL-17 also inhibited healthy LC migration. Finally, neutralising IL-17 in psoriasis skin resulted in enhanced LC migration. Collectively, these data suggest that an altered KC secretome, driven by increased expression of IL-17, is responsible for impaired LC migration in uninvolved skin of patients with psoriasis.

INTRODUCTION

Psoriasis is a common inflammatory skin condition affecting approximately 2% of the UK population. Langerhans cells (LC) are the dendritic cells of the epidermis. After encountering a pathogen or allergen, LC migrate from the epidermis to the lymph nodes where they regulate immune responses^{1,2}. Furthermore, epidermal LC also regulate immunity in the skin by interaction with T regulatory cells³. We have shown previously that in the uninvolved skin (PN) of patients with early-onset chronic plaque psoriasis (presenting before age 40) there is impaired LC migration *in vivo*⁴. Furthermore, we have replicated these findings

using an *ex vivo* epidermal explant culture model where healthy epidermis shows an approximate 20% reduction in LC compared with no reduction in LC frequency in PN⁵.

The interleukin (IL)-17/IL-23 pathway appears to be integral to the pathogenesis of psoriasis and monoclonal antibody therapies targeting these cytokines have proved to be highly successful^{6,7}. We have recently shown that IL-17 treatment completely inhibits LC mobilisation in both human and mouse skin *ex vivo*⁸. Interleukin-17 can act directly on keratinocytes (KC) resulting in up-regulation of cytokines and anti-microbial peptides⁹⁻¹². We have examined the effects of KC secreted factors from healthy skin and PN on epidermal LC migration. We also investigated the effect of IL-17 on healthy KC in addition to the effect of IL-17 neutralisation on LC migration in psoriasis skin.

METHODS

Volunteers and Skin Samples

Volunteers were aged between 18-65 years and were divided into the following groups: healthy with no history of skin disease (healthy) and volunteers with early-onset chronic plaque psoriasis (psoriasis). Psoriasis volunteers had not received any systemic or phototherapy for at least a month before participation. Volunteers provided up to four 6 mm punch biopsies, taken under local 1% lignocaine anaesthetic, from sun-protected buttock skin (in the case of psoriasis volunteers this was PN). The study was approved by the NRES Committee Northwest – Greater Manchester West Research Ethics Committee (13/NW/0867) and was conducted according to the Declaration of Helsinki and all subjects provided written, informed consent.

Keratinocyte Culture

Keratinocytes were isolated from 6 mm skin biopsies by a method described previously¹³, with slight modifications. Briefly, the skin was floated on dispase overnight at 4°C. The epidermis was isolated and floated on 1 ml TrypLE solution (Life Technologies) for 20 min at 37°C with slight agitation. The epidermis was removed and the TrypLE solution containing cells was neutralised using DMEM containing 10% foetal calf serum (FCS, both Life Technologies), strained through a 20 µm gauze and centrifuged at 200 g for 5 min. Cells were re-suspended in 1 ml of complete EpiLife media containing human KC growth supplement (Life Technologies), penicillin/streptomycin and amphotericin B (Sigma) and transferred to one well of a 12-well plate coated with collagen coating matrix (Life Technologies).

Generation of Keratinocyte Conditioned Medium and Lysates

To generate KC conditioned medium, cells were allowed to reach confluence in a 12 well plate and fresh complete EpiLife media was added to each well. In some experiments KC received addition of 100 ng/ml recombinant human IL-17 (R&D systems), or bovine serum albumin (BSA) vehicle control (Sigma). After 6 days media was harvested and centrifuged at 200 g and the conditioned medium stored at -80°C for future use in explant assays and for analysis by ELISA and Proteome Profiler Array. Keratinocytes were lysed from the plate using 350 µl per well of lysis buffer (Life Technologies) containing 1% β-mercaptoethanol and stored at -80°C for RNA extraction.

Epidermal Explant Assay

From each volunteer between 2 and 4 skin biopsies were collected and epidermal sheets removed as described previously⁵. One was fixed in acetone immediately (T0 control). The others were cultured by floating on 500 µl RPMI media (Life Technologies) containing 10%

FCS (RPMI/FCS) in a 24-well plate for 24 h (T24) before fixing. In some experiments, epidermal explants were cultured with previously generated KC conditioned medium. In these experiments complete EpiLife medium was used in place of RPMI for control cultured sheets. In some experiments 100 ng/ml recombinant IL-17 and/or 1-2 µg/ml neutralising goat anti-human IL-17 was added into the culture media (both R&D Systems).

Whole Skin Explant Assay

From each volunteer the epidermal sheet was removed from one biopsy and isolated and fixed (T0) as described previously⁵. Another biopsy from the same volunteer was floated on 500 µl RPMI/FCS in a 24-well plate for 24 h. For psoriasis volunteers, biopsies were also incubated with 2 µg/ml neutralising goat anti-human IL-17 antibody (or normal goat serum control) in 500 µl RPMI/FCS. After 24 h, epidermal sheets were isolated as described previously (T24).

Analysis of Epidermal Sheets

Epidermal sheets from explant assays were stained for LC using CD1a as a marker, as described previously⁵. Fifty fields were counted in the central portion of each epidermal sheet. The frequency of LC was expressed as cells per mm² and migration was examined as the percentage decrease in LC frequency in the T24 epidermal sheets compared with the T0 from the same volunteer, as described previously.

Proteome Profiler Array

Healthy and psoriasis KC conditioned media samples from multiple volunteers were pooled and analysed using Human XL Cytokine Proteome Profiler and the Human Chemokine Proteome Profilers (both R&D systems) according to the manufacturer's instructions.

ELISAs

Keratinocyte conditioned media were analysed for CCL20, IL-8 and matrix metalloproteinase (MMP)-9 by DuoSet ELISA (R&D Systems), performed according to the manufacturer's instructions.

Quantitative PCR

RNA was extracted from cell lysates using an RNA isolation kit, followed by cDNA conversion using a High Capacity RNA-to-cDNA kit (both Life Technologies) according to manufacturer's instructions. cDNA was used in quantitative real time PCR reactions using TaqMan primers (Applied Biosystems). Expression of genes of interest were measured relative to the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Fold change was calculated using the $\Delta\Delta C_t$ method. Results are presented as fold change in gene expression relative to the average healthy ΔC_t value.

RESULTS

Psoriasis KC Inhibit LC Migration in Healthy Skin

We have demonstrated previously that, in PN, LC migration is impaired^{4,5}. To examine whether healthy KC factors can restore LC migration in PN we cultured epidermal explants of PN using conditioned media generated from healthy KC (Figure 1). There was little or no LC migration in psoriasis samples in this model; the frequency of LC in the EpiLife media T24 group didn't differ significantly from the T0 control (Figure 1a). Healthy KC conditioned media had no effect on PN i.e. no significant difference in LC frequency was found between epidermal sheets cultured in EpiLife control or healthy KC conditioned medium (Figure 1b), and did not alter the percentage migration; $3.4\pm 4.5\%$ and $2.9\pm 3.7\%$ for EpiLife control and healthy KC conditioned medium respectively (Figure 1c).

Subsequently, we examined whether secreted factors from psoriasis KC altered LC migration in healthy skin by culturing healthy epidermis with conditioned media from psoriasis KC derived from PN. LC frequency in healthy skin was significantly lower than in the T0 control after 24 hours culture with EpiLife (630 ± 99 LC/mm² versus 781 ± 133 LC/mm²; Figure 1d) or healthy KC conditioned medium (632.8 LC/mm²; Figure 1e). LC migration levels were not significantly different between the EpiLife control and healthy KC conditioned medium cultured epidermal sheets (Figure 1g), and levels of LC migration were comparable with that previously seen in individuals with healthy skin⁵. However, when healthy epidermal sheets were cultured with psoriasis KC conditioned media, there was no reduction in LC frequency in the T24 epidermal sheet (Figure 1f) and the proportion of LC migrating was significantly reduced to $1.0\pm 1.2\%$ (Figure 1g).

Interleukin-17 Induces Changes in Healthy KC

As we have previously shown that the psoriasis-associated cytokine IL-17 inhibits LC migration in healthy skin^{8,14}, we sought to examine IL-17-induced changes in healthy KC. Keratinocytes from multiple donors were treated with IL-17 or BSA control and analysed for changes in genes associated with psoriasis. Data were expressed as the mean fold change in each sample against the mean value for the BSA control. Keratinocytes treated with IL-17 showed significantly up-regulated levels of DEFB4, IL-8 and CCL20 (Figure 2a; d and e) while MMP-9 expression was significantly decreased (Figure 2c).

We have shown previously that recombinant human IL-17 impairs LC migration in the explant model⁸. Therefore, we went on to investigate whether IL-17 can affect the ability of KC to inhibit LC migration in this model by culturing healthy epidermis with conditioned

media generated from IL-17 treated KC. To ensure that any effect of IL-17 on LC migration was due to the downstream effects of IL-17 on KC and not residual recombinant IL-17 in the KC conditioned media, we employed a neutralising IL-17 antibody in each of the epidermal explant culture conditions. In our previous study LC migration was completely inhibited ($0.9\pm 1.7\%$) when epidermal sheets were cultured with 100 ng/ml IL-17⁸. The same concentration of recombinant IL-17 was used in the current study to treat KC for generation of conditioned media, and to directly treat healthy epidermal explants in the presence of a neutralising IL-17 antibody as a control for the LC migration experiments. This control group had a lower frequency of LC compared with T0 (Figure 3c) and percentage LC migration levels in this group were in the normal range ($19.6\pm 10.5\%$; Figure 3d) demonstrating that the antibody used effectively neutralises this concentration of IL-17 in this model. When healthy epidermal sheets from the same individuals were incubated with conditioned media from KC treated with vehicle control (BSA; Figure 3a) there was a significant reduction in LC density equivalent to $22.9\pm 1.0\%$ migration (Figure 3d). However, when healthy epidermal sheets were incubated with conditioned media from IL-17 treated KC, LC frequency was reduced to a lesser extent and migration levels were reduced by half to $10.4\pm 7.7\%$ (Figure 3d). Thus the decrease in LC migration observed in the epidermal sheets cultured with IL-17-treated healthy KC conditioned media is a consequence of alterations in the KC secretome in response to IL-17 and not residual recombinant IL-17 in the conditioned media.

No Difference in IL-17 receptor Expression in Psoriasis KC from Uninvolved Skin

Next we examined the expression of genes directly related to the IL-17 pathway in psoriasis versus healthy KC. Firstly we examined levels of IL-17 receptor subunits but they did not differ between healthy and psoriasis KC (Figure 4a; b; c; d; e). To determine whether psoriasis KC were up-regulating and maintaining IL-17 downstream signalling pathways in

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response to increased exposure to IL-17 *in vivo*, we examined gene expression levels of CEBPB (gene encoding CCAAT/enhancer-binding protein- β ; Figure 4f) and NFKBIZ (gene encoding the protein NF-kappa-B inhibitor ζ ; Figure 4g), however there were no significant differences between the groups.

Conditioned media from healthy and psoriasis KC have similar cytokine profiles

As conditioned media from psoriasis KC inhibits LC migration, we wanted to examine the cytokine and chemokine profile. To do this we utilised an XL cytokine (Figure 5a) and a chemokine (Figure 5b) proteome profiler to analyse pooled healthy versus pooled psoriasis KC. There were no large differences in cytokine and chemokine production between healthy and psoriasis cells. To validate the results of the proteome profiler arrays, several proteins were selected for individual samples to be tested quantitatively by ELISA (MMP-9; Figure 5c, CCL20; Figure 5d, IL-8; Figure 5e, and CCL5; Figure 5f) but no significant differences were found.

Blocking IL-17 leads to improvements in LC migration in a whole skin explant model

As we have demonstrated that the changes in psoriasis KC responsible for impairing LC migration are likely driven by exposure to increased IL-17 *in vivo*, and as previous literature suggests that the source of IL-17 in psoriatic skin is dermal cells¹⁵, we next analysed whether addition of anti-IL-17 neutralising antibody to whole (epidermis and dermis) cultured PN would restore LC migration. Initially we analysed the level of LC migration in the healthy whole skin explant model and the mean percentage LC migration was 29.6% (Figure 6a). LC migration in whole PN varied from 0 to 19.2% but on average were significantly lower than those for healthy (11.2%; Figure 6a). When psoriasis explants were treated with NGS control alone, there was no significant reduction in LC density in the T24 epidermal sheet compared with the T0 control (Figure 6c). In contrast, when explants were cultured in the presence of

neutralising anti-IL-17 antibody, there was a significant reduction in LC density in the T24 epidermis (Figure 6d). When comparing the two groups as percentage LC migration, addition of neutralising anti-IL-17 antibody led to a significant improvement in LC migration levels (to 24.61%; Figure 6b).

DISCUSSION

Our current findings suggest that KC play an important role, and may be solely responsible, for the impaired LC migration observed in the PN skin in chronic plaque psoriasis. Our previous studies have shown that there is no obvious impairment of blood monocyte-derived LC¹⁶. The implication is rather that impairment of LC migration is associated with an altered epidermal phenotype. The data presented here are consistent with that view. Conditioned medium from psoriasis KC, but not from healthy KC, inhibited LC migration in an explant model incorporating normal epidermis. These data provide evidence, for the first time, that products of KC in PN sites of patients with psoriasis are able to impair LC migration.

As we know that IL-17 inhibits LC migration⁸, we sought to characterise the effects of IL-17 on KC, in order to establish whether IL-17 was responsible for driving the observed changes in psoriasis KC. We observed changes in response to IL-17, including elevated expression of DEFB4, the gene encoding β -defensin 2, and increased secretion of CCL20, consistent with previous studies^{9,17}. Over-expression of β -defensin 2 and CCL20; chemoattractants for CCR6, expressed by immature LC¹⁸⁻²⁰, may result in retention of LC within the epidermis. Treatment with IL-17 resulted in decreased secretion of MMP-9, a protease required for migration to the dermis through the basement membrane^{21,22} and thus a reduction in MMP-9 secretion could also contribute to impaired LC migration. We found here that treatment with

IL-17 not only changed gene and protein expression, but also altered the phenotype of healthy KC to function in a way comparable with psoriasis KC, that is, that conditioned media generated from IL-17-treated healthy KC inhibited healthy LC migration. This suggests that the altered function seen in psoriasis KC is a consequence of increased IL-17 in the skin. Recent data have shown that IL-17 downstream genes are also up-regulated in PN¹¹, confirming our findings that dysregulation of IL-17 signalling is also relevant in skin that appears clinically normal. It is unclear why PN skin appears clinically healthy despite responding to IL-17 and future studies should examine whether PN is simply exposed to less IL-17 compared with lesional skin, or whether there are regulatory mechanisms in place that prevent the formation of psoriasis plaques despite responsiveness to IL-17.

In order to examine whether psoriasis KC are more efficient at responding to IL-17, we examined the expression of IL-17 receptor subunits, however no significant differences were found between cells from healthy and psoriasis donors. The implication is that psoriasis KC do not have an increased capacity to respond to IL-17, and it is therefore more likely that altered function observed with psoriasis KC is simply due to more IL-17 being available in the skin. When investigating downstream mediators of IL-17 including CEBPB and NKZBIZ, genes which are up-regulated in psoriasis^{12,23,24}, no differences were found between cultured healthy and psoriasis KC, suggesting that psoriasis KC in culture are not actively responding to previous IL-17 exposure in the skin. Nevertheless, psoriasis KC still maintained a phenotype *ex vivo* that was capable of inhibiting LC migration in healthy epidermis. We suspect that psoriasis KC would not retain such a strong cytokine phenotype when cultured *ex vivo* compared with healthy KC that have recently been treated with a high dose of IL-17 *in vitro* as it is likely that the production of cytokines and chemokines by KC in response to IL-17 exposure is fairly transient. Indeed, when comparing the healthy and

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psoriasis KC secretome we failed to see any differences in cytokine and chemokines tested, including those up-regulated by healthy KC in response to IL-17. The interpretation is, therefore, that although the IL-17-induced up-regulation of chemoattractants (such as β -defensin 2 and CCL20) and down-regulation of MMP-9 may contribute to the impaired LC mobilisation observed *in vivo*, it is not a cytokine or chemokine that is responsible for directly inhibiting LC migration in this model. The retained phenotype of psoriasis KC to impair LC migration long after exposure to IL-17 could be due to alterations in the secretome of other classes of proteins or even molecules such as lipids. Future work is required to determine the factor, or factors, secreted by psoriasis KC, that impair LC migration, and furthermore how they are regulated by IL-17.

Although we have previously shown that IL-17 directly impairs healthy LC migration ⁸, and we report here that IL-17 induces healthy KC to secrete a factor that inhibits LC migration, there was a need to confirm that up-regulation of IL-17 in PN skin is the mechanism responsible for impaired LC function. We found that neutralisation of IL-17 resulted in the restoration of normal LC mobilisation in PN, confirming that an IL-17-driven mechanism is responsible for altering LC function in psoriasis. We have shown previously that anti-cytokine treatments restore LC migration in psoriasis patients ⁵. Further work should examine whether LC migration is restored in the skin of patients using anti-IL-17 therapies. As LC play a key role in orchestrating immune responses ³, one theory is that the decreased ability of LC to migrate from the skin to the lymph node may result in a lack of regulation of the immune response. It is currently unknown whether the function of LC is also impaired in psoriasis plaques, but one study has demonstrated that there are increased numbers of activated LC found at perilesional sites ²⁵, suggesting that LC migration may also be impaired here. Together our data have shown that the observed impairment in LC migration in the PN skin

of chronic plaque psoriasis is due to IL-17-driven changes in the phenotype and function of KC, leading to changes in the KC secretome that impair LC function.

FIGURE LEGENDS

Figure 1. Conditioned media from psoriasis keratinocytes inhibits Langerhans' cell migration in a healthy epidermal explant model

Conditioned media was generated from healthy (H) and psoriasis (PS) keratinocytes (KC) (n = 4 per group). Biopsies were obtained from uninvolved psoriasis skin and the epidermis was isolated and fixed immediately (T0) or cultured for 24h. The mean density of Langerhans' cells (LC) in T24 epidermal sheets cultured with (a) EpiLife or (b) healthy KC conditioned media, compared with T0, n = 7. Epidermal sheets were prepared from two biopsies from healthy individuals and one was fixed immediately (T0) and the other cultured for 24 h. The mean density of LC in T24 epidermal sheets cultured with (d) EpiLife, (e) healthy KC conditioned media and (f) PS KC conditioned media compared with T0, n = 6 per group. The significance of differences between the T0 and the T24 samples was analysed by paired *t* test. The percentage migration levels in the T24 epidermal sheet compared with the T0 for each group for psoriasis explants (c) and healthy explants (g). The statistical significance of differences was analysed by *t* test (c) or one-way ANOVA (g), *** $p < 0.01$.

Figure 2. Interleukin-17 alters gene expression and protein secretion by healthy keratinocytes

Healthy keratinocytes (KC) were treated with bovine serum albumin (BSA) and 100 ng/ml interleukin (IL)-17 for 6 days. Cells were lysed and supernatants harvested. RNA was isolated and converted to cDNA which was used for PCR analysis. The ΔC_t of each gene of interest was calculated against the GAPDH housekeeping gene for each sample. The $\Delta\Delta C_t$

method was used to calculate mean fold change for each sample compared to the average ΔCt value for the BSA samples. The mean fold change for BSA and IL-17 samples for (a) DEFB4 and (b) S100A7. Supernatants were analysed for (c) matrix metalloproteinase (MMP)-9, (d) CCL20 and (e) IL-8 by ELISA. Each line represents matched BSA and IL-17 for each KC sample, $n = 6$. The statistical significance of differences between BSA and IL-17 groups was analysed by paired t test, * $p < 0.05$, ** $p < 0.01$.

Figure 3. Conditioned medium from healthy keratinocytes treated with interleukin-17 inhibits Langerhans' cell migration in healthy epidermal explants

Conditioned media was generated from healthy keratinocytes (KC) treated with 100 ng/ml interleukin (IL)-17 and bovine serum albumin (BSA) vehicle control ($n = 3$). Four biopsies were taken from healthy volunteers and epidermal sheets were isolated. One was fixed immediately and two were cultured with conditioned media from either BSA-treated or IL-17-treated KC, plus 1-2 $\mu\text{g/ml}$ neutralising anti-IL-17 antibody (to ensure that any effects observed were due to downstream effects of IL-17 on KC rather than the residual IL-17 remaining in the conditioned media from KC treatment). The final biopsy was incubated with 100 ng/ml recombinant IL-17 with the same concentration of neutralising antibody (1-2 $\mu\text{g/ml}$), to ensure that the antibody sufficiently inhibited the effects of IL-17 on LC migration. The frequency of LC in epidermal sheets in T0 control compared with T24 sheets cultured with (a) BSA treated KC conditioned media, (b) IL-17-treated KC conditioned media and (c) recombinant IL-17 control. (d) The percentage LC migration in the T24 groups compared with the T0 group. The statistical significance of differences was analysed by paired t test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 4. Psoriasis keratinocytes have no change in interleukin-17 receptor expression but have lower levels of interleukin-17C

Healthy (H) and psoriasis (PS) keratinocytes (KC) were cultured and cells were lysed (n = 7 per group). RNA was isolated and converted to cDNA which was used for PCR analysis. The ΔCt of each gene of interest was calculated against the GAPDH housekeeping gene for each sample. The $\Delta\Delta\text{Ct}$ method was used to calculate mean fold change for each sample compared to the average healthy ΔCt value for the same gene. The mean fold changes are displayed for (a) IL-17RA, (b) IL-17RB, (c) IL-17RC, (d) IL-17RD, (e) IL-17RE, (f) CEBPB and (g) NFKBIZ. The statistical significance of differences between groups was analysed using an unpaired *t* test, * $p < 0.05$.

Figure 5. Healthy and psoriasis keratinocytes have comparable cytokine and chemokine secretion

Conditioned media from healthy and psoriasis keratinocytes (KC) was pooled (n = 4 per group) and analysed for cytokines and chemokines using (a) a cytokine proteome profiler and (b) a chemokine proteome profiler. Each pair of dots indicates detection of a cytokine/chemokine. Conditioned media was analysed for (c) matrix metalloproteinase (MMP)-9, (d) CCL20, (e) interleukin (IL)-8 and (f) CCL5 by ELISA (n = 5 per group). The dotted line indicates the accurate level of detection of the ELISA.

Figure 6. Langerhans' cell migration in psoriasis whole skin explants is lower than healthy explants and is improved with neutralising anti-interleukin-17 antibody

Biopsies were taken from healthy and psoriasis individuals. For one biopsy the epidermis was removed and fixed immediately. The second biopsy was cultured for 24 h in either media alone, or for psoriasis biopsies 2 $\mu\text{g/ml}$ neutralising anti-interleukin (IL)-17 antibody or normal goat serum (NGS) vehicle control before the epidermis was removed and fixed. (a)

Percentage Langerhans' cell (LC) migration in T24 healthy and psoriasis epidermal sheets compared with the concurrent T0 control. (b) Percentage LC migration in psoriasis samples cultured with either NGS control or anti-IL-17 neutralising antibody. (c) Mean LC density in psoriasis skin cultured with NGS compared with T0 and (d) cultured with anti-IL-17. The statistical significance of differences was analysed by unpaired (a) and paired (b, c and d) *t* tests ** $p < 0.01$, *** $p < 0.001$.

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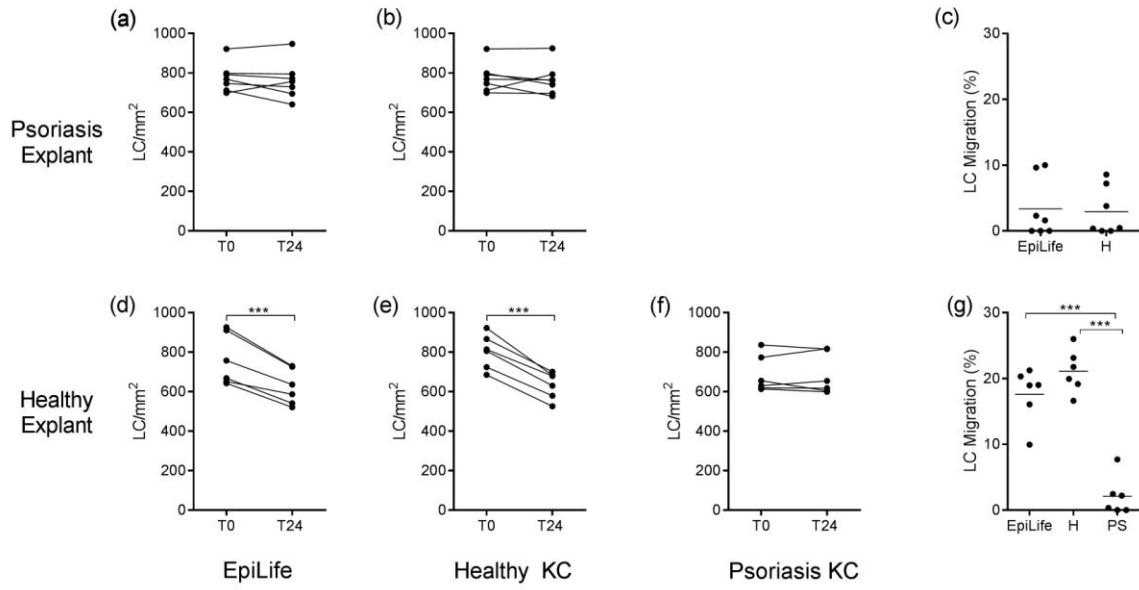
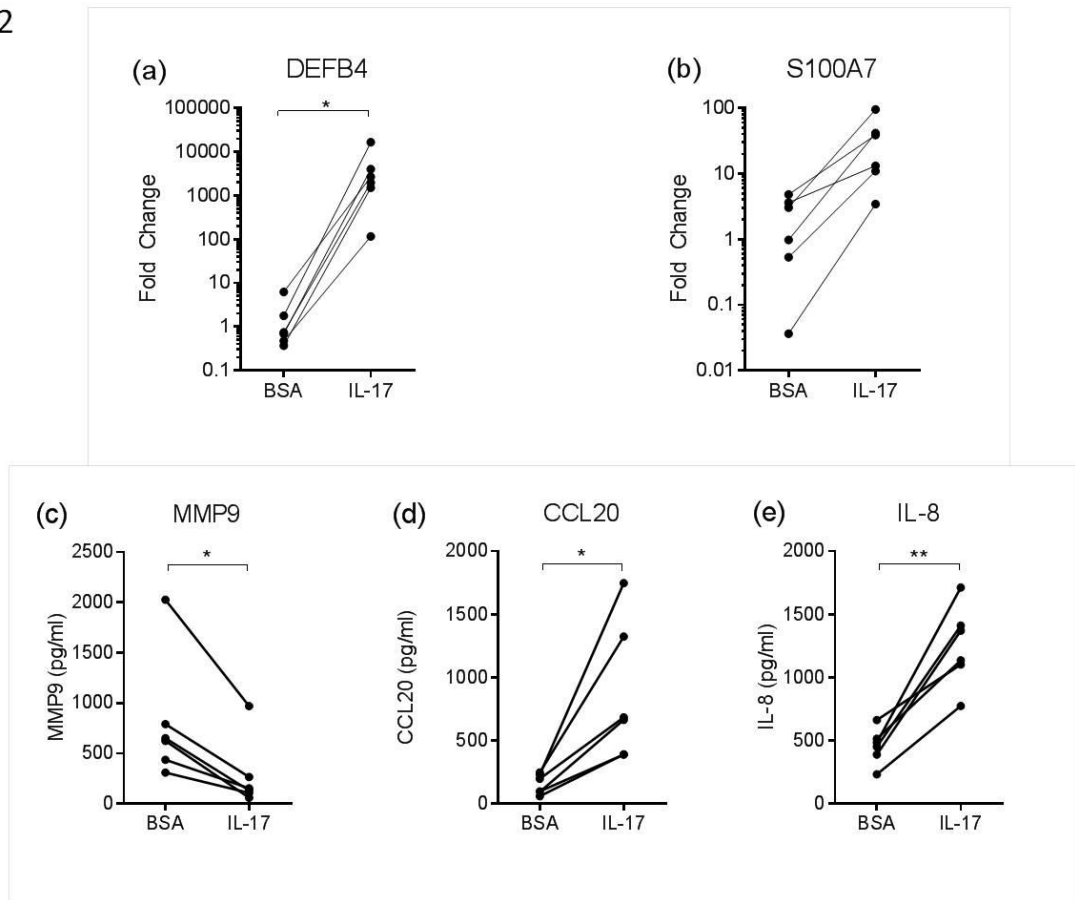


Figure 2



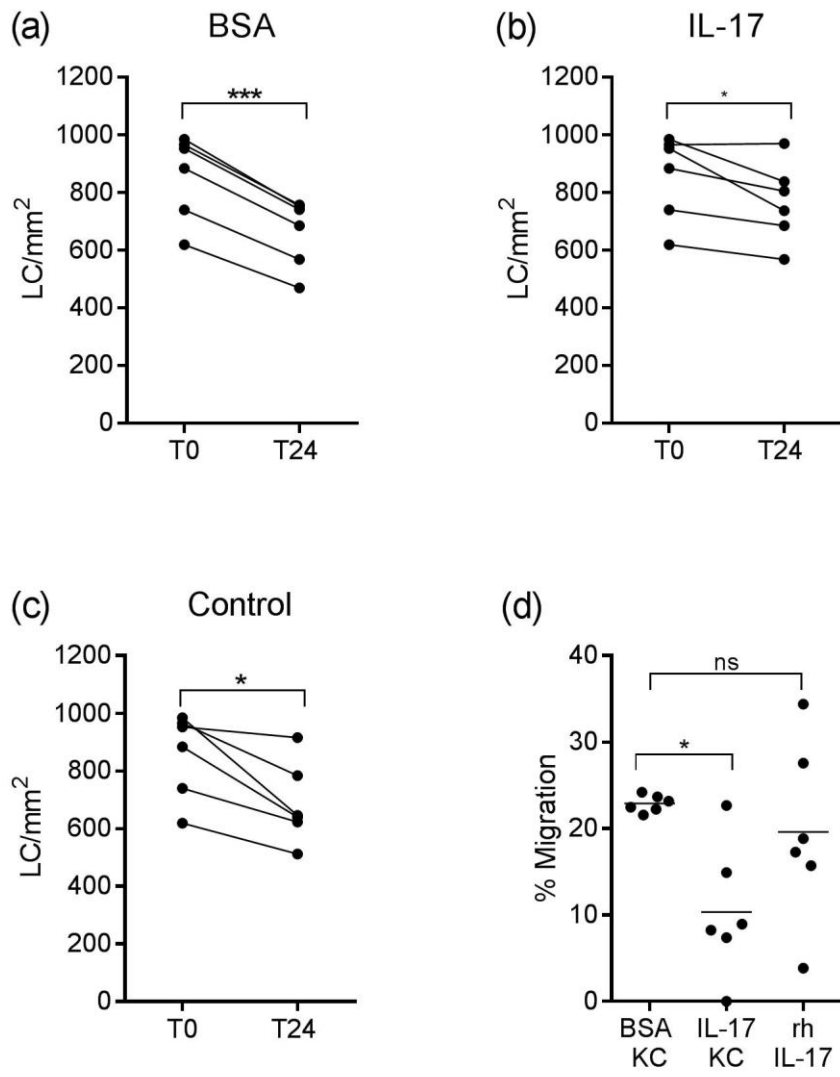


Figure 4

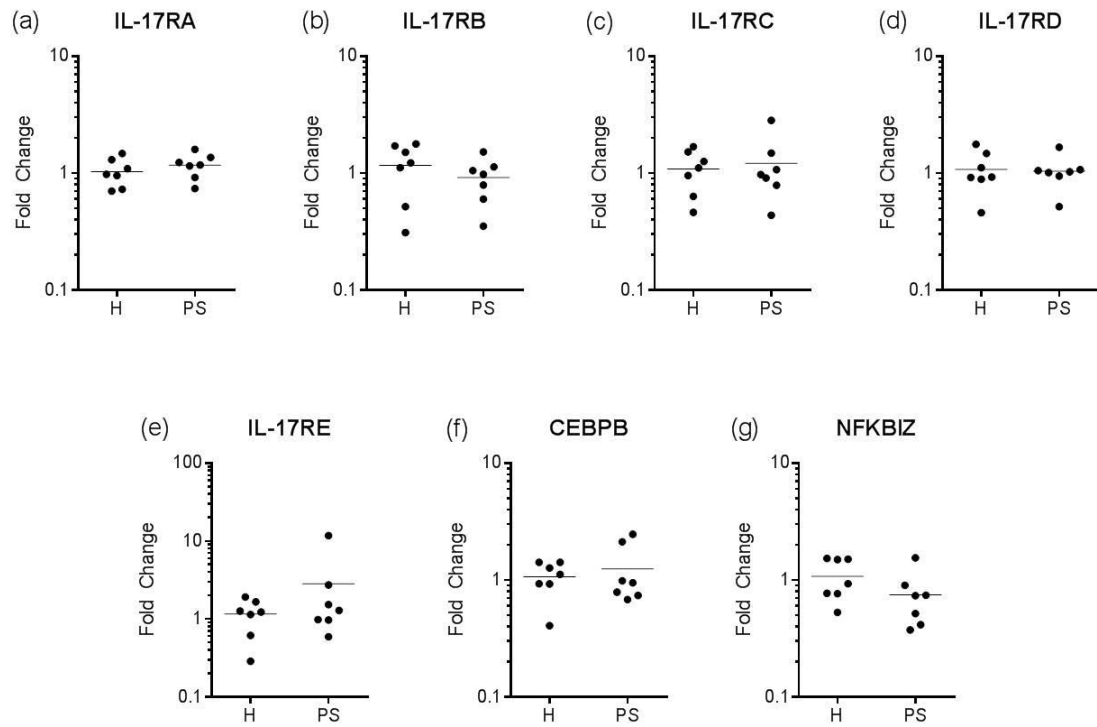


Figure 5

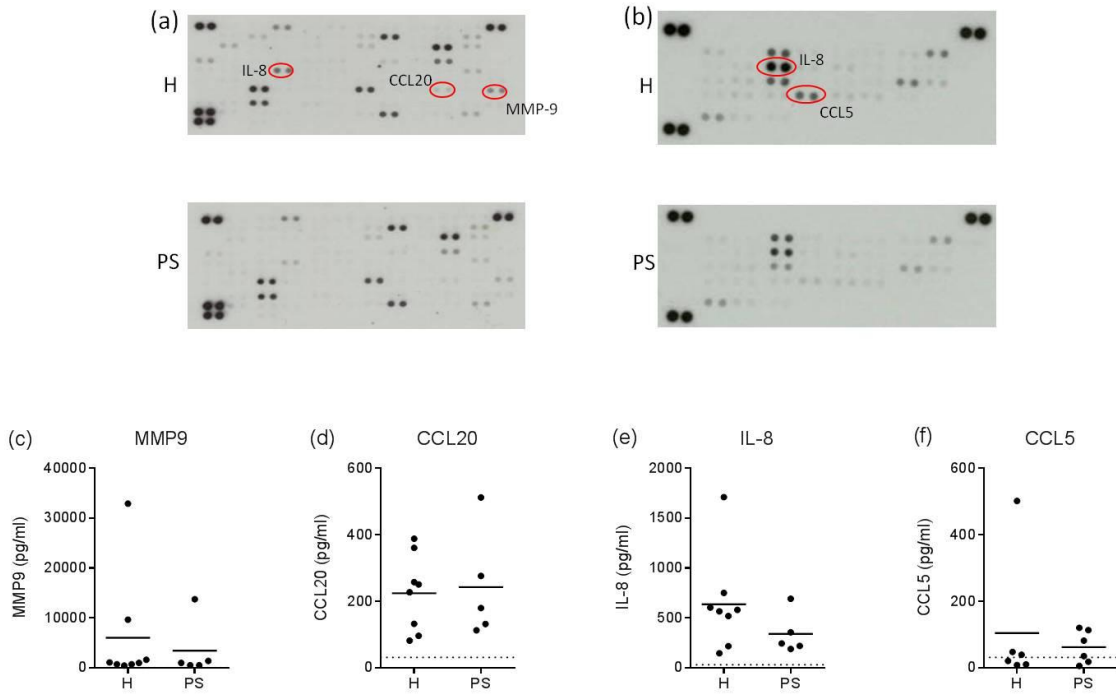


Figure 6

