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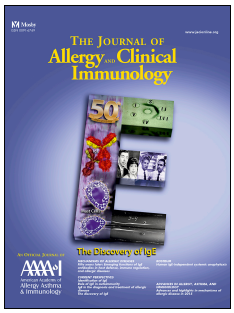

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β-defensin-2 is a responsive biomarker of IL-17A-driven skin pathology in psoriasis

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ABSTRACT

Background: IL-17A is a key driver of human autoimmune diseases, particularly psoriasis.

Objective: We sought to determine the role of IL-17A in psoriasis pathogenesis, and to identify a robust, measurable biomarker of IL-17A-driven pathology.

Methods: We studied 8 healthy and 8 psoriasis subjects before and after administration of secukinumab, a fully human anti-IL-17A mAb, and utilized a combination of classical techniques and a novel skin microperfusion assay to evaluate the expression of 170 proteins in blood, non-lesional skin and lesional skin. For validation, we also tested stored sera from 601 subjects with a variety of autoimmune diseases.

Results: IL-17A was specifically expressed in lesional compared to non-lesional psoriatic skin (9.8 vs. 0.8pg/ml, $p<0.001$). Proteomic and gene transcription analyses revealed dysregulated anti-microbial peptides, pro-inflammatory cytokines and neutrophil chemoattractants, which returned towards normal after treatment with secukinumab. β-defensin-2 (BD-2) was identified as a biomarker of IL-17A driven pathology by comparing protein expression in psoriasis vs healthy states (5746 vs. 82pg/ml in serum, $p<0.0001$; 2747 vs. <218pg/ml in dermis, $p<0.001$), responsiveness to secukinumab therapy, and synergistic induction by IL-17A and TNFα in epidermal keratinocytes. In a validation set of sera from 601 subjects with autoimmune diseases thought to be IL-17A-driven, we found that BD-2 is most highly elevated in subjects with psoriatic skin lesions, and in psoriasis correlated well with IL-17A levels ($r=0.70$, $n=199$, $p<0.001$) and PASI ($r=0.53$, $n=281$, $p<0.001$).

Conclusion: IL-17A is a primary driver of skin pathology in psoriasis, and serum β-defensin-2 is an easily measurable biomarker of IL-17A driven skin pathology.

Clinical Implications: Serum β-defensin-2 may be a valuable biomarker to predict and/or monitor response to IL-17A based therapies.

Capsule Summary: In psoriasis, the IL-17A inhibitor secukinumab reduced anti-microbial peptides, pro-inflammatory cytokines and neutrophil chemoattractants as measured by skin and serum proteomic profiling, and identified serum β-defensin-2 as an easily measurable biomarker of IL-17A driven skin pathology.

Key Words: IL-17; psoriasis; secukinumab; β-defensin-2; biomarker; dermal interstitial fluid; microperfusion; psoriatic arthritis; ankylosing spondylitis; rheumatoid arthritis; multiple sclerosis; autoimmunity

Abbreviations: AS, ankylosing spondylitis; BD-2, β-defensin-2; CD, Crohn's disease; dISF, dermal interstitial fluid; dOFM, dermal open flow microperfusion; GeoMean, geometric mean; HV, healthy volunteer; IL, interleukin; LLOQ, lower limit of quantification; MS, multiple sclerosis; PASI, psoriasis area and severity index; PsA, psoriatic arthritis; Pso, psoriasis; RA, rheumatoid arthritis; sc, subcutaneous.

INTRODUCTION

Th17 cells and the interleukin (IL)-17 family of cytokines are key drivers of autoimmune diseases such as psoriasis (Pso), psoriatic arthritis (PsA), ankylosing spondylitis (AS), rheumatoid arthritis (RA) and multiple sclerosis (MS) [1]. Psoriasis is the best studied of these diseases with multiple interventions targeted at Th17 cells and their cytokines showing efficacy in its treatment.

The IL-17 family of cytokines includes six members (IL-17A-F) that function as homo- or hetero-dimers to signal through a family of cytokine receptors with multiple chains (IL-17RA-E) with IL-17A and IL-17F signaling through a receptor complex composed of IL-17RA and IL-17RC [2, 3]. Circulating levels of IL-17A protein have been shown to be higher in psoriasis patients than in healthy controls and correlate with disease severity [4], while mRNA expression of *IL17A, IL17C*, and *IL17F* genes is higher in psoriatic lesional tissue than in non-lesional tissue [5-7]. Indeed, therapies that target either IL-17A or IL-17RA are similarly highly effective in reducing disease in psoriasis [1, 8-11]. While these numerous lines of evidence implicate an important role for IL-17A, a non-redundant role for IL-17F has not been precluded.

Much of our current understanding of psoriasis pathophysiology and the effects of IL-17A blockade has largely been based on studies evaluating gene expression from skin biopsies [6, 12-14]. While these studies have provided significant insights into the pathways that may be involved in IL-17A mediated pathogenesis, they are limited by the frequent incongruence between changes in mRNA and protein expression. Furthermore, they do not quantify the early micro-anatomically specific changes in protein expression, in particular large proteins such as cytokines and chemokines. To date, protein expression response to therapy in psoriatic skin has been mostly demonstrated on a qualitative rather than quantitative level through the use of histology and immunohistochemistry [12, 14-16].

To increase our understanding of the inflammatory pathophysiology in psoriatic skin at a protein expression level and to identify a protein biomarker of IL-17A mediated pathology, we conducted an exploratory study in eight healthy and eight psoriatic subjects to quantify and compare the soluble proteomic profiles of lesional versus non-

lesional before and after single dose systemic treatment (300 mg subcutaneous) with secukinumab (a fully human anti-IL17A monoclonal antibody with a half-life of ~27 days), and/or healthy skin. Dermal open flow microperfusion (dOFM), a novel technique that provides minimally invasive access to the dermal interstitial fluid (dISF), was used to sample the dermis [17-19]. The dermal (dISF) samples were then analyzed for 170 proteins encompassing cytokines, chemokines, growth factors, cell adhesion molecules and soluble receptors. Early proteomic changes that occur within the dermis following secukinumab treatment were complemented with gene expression changes extracted from full skin biopsies. Our aim was to identify which pathways might be central to psoriasis pathology in skin, and whether the anti-microbial peptide β-defensin-2 (BD-2) may be a protein biomarker of IL-17A mediated pathology.

METHODS

Study design

This single-center clinical study was conducted according to ethical principles and good clinical practice at the Medizinische Universität in Graz, Austria, after being approved by the ethic committee and Austrian health authority. Enrolled patients needed to sign informed consent and comply with the protocol requirements (ClinicalTrials.gov Identifier NCT01539213).

We enrolled 8 healthy subjects (mean age 26.1 years) and 8 subjects with psoriasis (mean age 38.8 years). All 16 subjects were Caucasian and all subjects with psoriasis were male, as were 6 healthy subjects, and all received a single 300 mg subcutaneous (sc) injection of secukinumab on study Day 1. Skin biopsies (4-mm skin punch), serum samples and dermal sampling were performed on study days 8 and 15 (7 and 14 days after secukinumab treatment). Details of the dermal sampling methodology were previously described [19].

In addition, baseline serum from patients with various inflammatory diseases sampled in multicenter trials, conducted after ethics committees and health authority approval, have been analyzed. Sera analyzed were from 289 patients with Pso (NCT01539213 and NCT00941031), 37 with PsA (NCT00809614), 56 with AS (NCT00809159), 68 with MS (NCT01051817), 94 with RA (NCT01426789), and 57 with CD (NCT00584740).

Protein and RNA measurements

Free IL-17A and IL-17F in serum and dISF were quantified using microparticle-based fluorescent sandwich immunoassays based on Erenna technology validated in human serum (Singulex® IL-17A Human Immunoassay kit, Cat No.: 03-0017-05; Singulex® IL-17F Human Immunoassay kit; Cat. No.: PN: 03-0018-03). The lower limit of quantification (LLOQ) for IL-17A was 0.64pg/ml in dISF and 0.096pg/ml in serum, and for IL-17F was 96.6pg/ml in dISF and 14.4pg/ml in serum. BD-2 was quantified by ELISA (Alpha Diagnostic, Cat No.: 100-250-BD-2). The LLOQ for BD-2 was 218pg/ml in dISF and 32.5pg/ml in serum. Sinistrin served as a reference substance and was used to estimate the absolute concentrations of biomarkers in dISF [19].

The chemiluminescent multiplex enzyme immunoassay platform from Aushon BioSystems (Billerica, MA, USA) was used to profile and quantify the levels of 170 proteins distributed over 43 panels. The panel of 170 proteins encompasses cytokines, cytokines receptor, chemokines, cell adhesion molecules, angiogenesis factors, matrix metalloproteinases, growth factors and neurotrophic factors (supplementary Table 1).

Total RNA was isolated from skin biopsies using the Qiagen RNeasy Micro Kit. RNA isolated from commercial skin biopsies (Asterand UK Limited) from healthy subjects (n=10) served as controls for gene expression data. Gene expression analysis was done by quantitative qRT-PCR or Nanostring technology. See Supplementary materials for further details.

Cell culture.

Human primary skin cells were obtained from PromoCell (Heidelberg, Germany). Epidermal keratinocytes (adult, pooled donors, passage five, 30,300 cells/cm^2), dermal fibroblasts (adult, single donor, passage six, 30,300 cells/cm^2) and dermal microvascular endothelial cells (juvenile, pooled donors, passage five, 22,700 cells/cm^2) were seeded into 96-well plates and incubated overnight. Thereafter, cells were incubated for another 20h in medium alone or 0.03-960ng/ml IL-17A in the absence or presence of 1ng/mL TNFα, 0.03-960ng/ml IL-17F in the absence or presence of 1ng/mL TNFα or 0.03-960ng/ml TNFα. Supernatants were examined for the release of BD-2.

Statistical analysis

For the skin micro-perfusion study, log-transformed baseline (BL, Day 1) IL-17A and IL-17F protein levels were analyzed by a mixed effects model with site (healthy volunteer or patient for serum levels, and lesional or non-lesional psoriatic skin for skin levels) as a fixed effect and subject as a random effect, respectively. The adjusted geometric means (GeoMean) at each site were provided on the original scale together with the p-values for comparisons between sites. Pearson's correlation coefficients (denoted by r) between baseline protein levels (IL-17A and IL-17F) and efficacy scores (PASI) were calculated together with the corresponding p-values by site. Log-transformed baseline BD-2 levels in serum and in skin were also analyzed by a mixed effects model with site (healthy volunteer or patient for serum levels and healthy, lesional or non-lesional

psoriatic skin for skin levels) and subject as a random effect, respectively. The adjusted GeoMeans at each site were provided on the original scale together with the p values for comparisons between sites. IL-17F levels in serum in psoriasis patients and BD-2 levels over time in serum and in skin were log-transformed and analyzed by site by a mixed effects model with visit day (BL, Day 8 and Day 15) as a fixed effect and subject as a random effect, respectively. The adjusted geometric means and 95% confidence intervals at each visit were provided on the original scale together with the p values for comparisons between visits. Pearson's correlation coefficients between BD-2 levels and efficacy scores (PASI) were calculated together with the corresponding p-values by site and visit. Any values below LLOQ (Note: values below LLOQ differ for some samples due to a different sample dilution factor applied) were imputed by half LLOQ.

In the secukinumab dose regimen study in patients with moderate to severe psoriasis (NCT00941031), serum samples were taken and analyzed for BD-2, IL-17A AND IL-17F. Correlations between PASI, BD-2, IL-17A and IL-17F were investigated using a Pearson's correlation coefficient. BD-2, IL-17A and IL-17F values were log transformed prior to analysis. Values below the LLOQ were not included in the calculations.

Analyses were performed using PROC MIXED and PROC CORR in SAS version 9.4.

RESULTS

Baseline IL-17A protein levels are increased in lesional versus non-lesional psoriasis skin

In order to understand the relative roles of IL-17A and IL-17F in psoriasis, we evaluated protein levels in the circulation (serum) and skin (dISF) of the 16 subjects in our study NCT01539213. Consistent with previous studies, baseline serum levels of IL-17A protein were higher in subjects with psoriasis than in healthy subjects (GeoMean 0.5 vs. 0.2pg/mL, respectively; $p<0.05$). Post-treatment serum levels of free IL-17A could not be assessed as the IL-17A assay used measures not only free IL-17A, but also IL-17A bound to secukinumab. No validated assay exists to measure free IL-17A in presence of secukinumab. Serum IL-17F levels in psoriasis patients (17.5pg/mL) did not change significantly at Day 15 from baseline after secukinumab (14.9pg/mL, p=NS). At baseline, serum IL-17A protein levels correlated well with PASI (psoriasis area and severity index; $r=0.93$, $p<0.001$; Figure 1A), while baseline IL-17F levels did not ($r=0.01$, $p=0.98$; Figure 1B).

Dermal (dISF) levels of IL-17A protein at baseline were significantly higher in lesional than in non-lesional psoriatic skin (GeoMean 9.8 vs.0.8pg/mL, respectively; $p<0.01$; Figure 1C). IL-17A was not detectable in the normal skin of healthy subjects (below the lower limit of quantification [LLOQ]; <0.64pg/mL). In contrast, baseline levels of IL-17F in dISF were not significantly different between lesional and non-lesional skin, although IL-17F levels tended to be higher in lesional than non-lesional skin (GeoMean 317 vs.163pg/mL, respectively; $p=0.105$, Figure 1C). IL-17F protein was not measurable in the normal skin of healthy subjects (below the LLOQ of 96.6pg/mL; Figure 1C). Transcriptional analysis of IL-17 family members (IL-17A-F) in skin biopsies confirmed these findings by demonstrating upregulation of mRNAs for IL-17A, IL-17F, IL-17C and potentially IL-17E (IL-25) in psoriatic lesional skin compared to healthy skin while expression of IL-17B was unchanged and that of IL-17D was reduced (Supplementary Figure S1). Consistent with changes in protein levels, *IL17A and IL17F* mRNA levels were reduced after secukinumab treatment (Supplementary Figure S1).

Proteomics screening of serum and dISF for IL-17A responsive markers

To identify potential markers of IL-17A response proteins, we employed a hypothesis-free proteomics screening approach measuring 170 proteins in dISF and serum before and after secukinumab treatment. Using a 1.5 fold-change from baseline as a threshold, 89 proteins were shown to be dysregulated in dISF and/or serum after treatment with secukinumab (Figure 2). The 10 proteins showing the greatest fold-decrease were anti-microbial peptides (β-defensin2 [BD-2] and lipocalin 2 [NGAL or LCN2]), metalloproteinases (MMP-1 and MMP-8), IL-1 pathway members (IL-1β and IL-1RA), neutrophil proteins (myeloperoxidase or MPO), and neutrophil (GRO-α or CXCL1, ENA-78 or CXCL5) or Th17 cell (CCL20) attracting chemokines (Table 1). Other chemokines including CXCL3 and CCL1 were also decreased after secukinumab. We could not detect a change in TNFα levels. The 5 proteins showing the greatest fold-increase were Th2 cell associated proteins (IgE and eotaxin2/CCL24), acrp30, leptin and endoglin. An early downregulation of anti-microbial peptides BD-2 (encoded by the gene *DEFB4A*), BD-3 (*DEFB103B*), NGAL (*LCN2*), LL37 (*CAMP*), *S100A8*, and *S100A9* as well as neutrophil and Th-17 attracting chemokines (*CXCL1*, *CXCL8* and *CCL20*, respectively), and IL-1 family members IL-36α (*IL36A*), IL-36β (*IL36B*), IL-36γ (*IL36G*) and IL-36RN (*IL36RN*) was also observed on the mRNA level (Supplementary Figures S2-S4), thereby confirming and extending observations made on the protein level. β-defensin 2 (BD-2, also known as skin antimicrobial peptide 1 or SAP1) was the protein showing the highest fold change in both dermis and serum and was selected for further study.

BD-2 levels correlate with IL-17A and psoriasis clinical severity, and decrease after IL-17A blockade

BD-2 levels were further and more specifically quantified by ELISA in skin (dISF) and serum before and after secukinumab treatment (Figure 3). At baseline, serum levels of BD-2 protein were significantly higher in subjects with psoriasis than in healthy subjects (GeoMean 5746 vs. 82pg/mL respectively, $p<0.001$; Figure 3). Serum levels of BD-2 decreased rapidly in subjects with psoriasis after a single 300 mg s.c. dose of secukinumab (GeoMean 971pg/mL at Day 8, and 649pg/mL at Day 15; $p<0.0001$ vs.

baseline for both time points). Dermal (dISF) BD-2 levels were also significantly higher in lesional skin than in non-lesional psoriatic skin at baseline (GeoMean 2747pg/mL vs. 417pg/mL, respectively, $p<0.001$; Figure 3). In contrast, dermal BD-2 levels in healthy subjects were all below the LLOQ ($p<0.001$ vs lesional skin in psoriasis subjects; Figure 3). After secukinumab treatment, mean BD-2 levels in lesional skin decreased by 80% at Day 8 and approached levels close to the LLOQ at Day 15 post-treatment with secukinumab (GeoMean 550pg/mL at Day 8, $p<0.05$; and 196pg/mL at Day 15, $p<0.01$; Figure 3). Thus, BD-2 in both serum and dISF is responsive to IL-17A inhibition.

To confirm that IL-17A mediates BD-2 expression in skin, we tested cytokine-stimulated BD-2 expression in epidermal keratinocytes, dermal fibroblasts and dermal microvascular endothelial cells. Of the three cell types, only epidermal keratinocytes produced BD-2 (Figure 4). While IL-17A, IL-17F and TNFα all induced low levels of BD-2 at high concentrations; the combination of IL-17A and TNFα was synergistic and induced high levels of BD-2 expression. Thus, epidermal keratinocytes are the IL-17A responsive skin cell type for BD-2 production.

To determine whether BD-2 could be a marker of psoriasis disease activity, we compared BD-2 serum levels with PASI scores. Clinical efficacy of secukinumab has been previously documented, and similarly in this study, the mean PASI score decreased by 62.5% at Day 22 after a single dose of secukinumab 300mg s.c. ($p<0.0001$ vs. baseline). BD-2 levels also declined in correlation with reductions in clinical disease severity (PASI scores at baseline, Day 8, and Day 15; $r = 0.87$-0.89; $p<0.01$). These data were confirmed by analyzing stored baseline serum samples from a second, larger Phase 2b study in psoriasis (NCT00941031, [20]). In this validation cohort, there was a significant correlation between serum BD-2 and IL-17A ($n=199$; $r=0.70$; $p<0.001$; Figure 5) as well as serum BD-2 levels and PASI disease activity ($n=281$; $r=0.53$; $p<0.001$; Figure 5). Thus, serum BD-2 levels correlate with both serum IL-17A and psoriasis disease activity. Furthermore, and in line with the close correlation of serum levels of BD-2 with IL-17A, serum levels of IL-17A show a significant correlation with psoriasis disease activity ($n=206$; $r=0.44$; $p<0.001$; Figure 5). Little correlation was

observed for IL-17F with either psoriasis disease activity (n=177; r=0.15; p<0.05; Figure 5) or serum BD-2 (n=173; r=0.15; p<0.05; Figure 5).

Since IL-17A is elevated and a pathogenic driver in multiple autoimmune diseases, we sought to determine if BD-2 could be a marker of IL-17A activity in diseases other than psoriasis. We analyzed BD-2 levels in stored baseline serum (n=601) from previous clinical studies in several autoimmune diseases. BD-2 was highly elevated in the serum of subjects with diseases having skin involvement such as psoriasis and PsA, and only at moderate levels in diseases with minimal to no skin involvement such as AS, RA, MS and CD (Figure 6). Although previous or current psoriatic inflammation of the skin is required for the diagnosis of PsA, many patients with PsA do not have detectable skin lesions at the time of evaluation. Thus, we evaluated whether BD-2 levels were different in subjects with or without ongoing skin involvement and found that BD-2 levels were higher in those patients with active skin involvement compared to those who did not (GeoMean 1493pg/ml vs 454pg/ml, p<0.01). We also assessed IL-17A and IL-17F levels in psoriasis, PsA and AS, and found that they were expressed at similar levels with or without skin involvement (Figure 6). Thus, BD-2 appears to be a biomarker of IL-17A pathogenesis in skin.

Discussion

In this study, our aims were to: (1) better understand the role of IL-17A in psoriasis pathogenesis by defining the early effects of IL-17A blockade on inflammatory proteins in both serum and the dermis; and (2) identify soluble biomarkers of IL-17A pathway activation.

Previous studies quantifying skin gene expression by transcriptomics or qualitatively assessing protein expression by IHC before and after anti-IL-17A therapy have provided important information [12, 13], but the conclusions are limited by the often discrepant timing and levels of gene and protein expression. This may be of particular relevance since proteins are the final effector molecules in biological systems and significant differences to their corresponding mRNA levels may exist [21]. To quantify soluble skin proteins involved in psoriasis pathogenesis, we used dermal open-flow microperfusion (dOFM) [18].

As predicted by IHC and mRNA transcription data, we found high levels of IL-17A protein in lesional skin from patients with psoriasis. Similarly and consistent with previous [12, 13] and our own mRNA transcription data, we could not detect either IL-17A or IL-17F protein in skin of healthy volunteers. Interestingly, we found detectable albeit lower IL-17A protein levels in non-lesional skin of psoriasis patients (2 out of 8 tested), suggesting that non-lesional skin is not normal and has a low level of ongoing IL-17A pathway activation. In the serum, we confirmed previous data, now using a highly sensitive and validated assay, that IL-17A in our small study of 8 subjects is elevated in psoriasis and correlates with disease activity. While IL-17F protein levels in psoriasis were much higher than IL-17A, they were not consistently upregulated in lesional versus non-lesional skin in all psoriasis plaques and did not correlate with psoriasis disease activity as measured by PASI. One possible explanation for this discrepancy is that IL-17A is about 100-fold more potent than IL-17F for signaling through IL-17R [22], a feature also seen for BD-2 induction in keratinocytes in Figure 4. We sought to validate this finding using a larger, independent cohort of patients with psoriasis. In the validation set, serum IL-17A continued to have a good correlation while IL-17F had a weaker if not poor correlation with PASI. Combined with the excellent and similar efficacy of compounds that target IL-17RA (which blocks IL-17A, IL-17C, IL-17E and IL-17F) and IL-17A only (reviewed in Patel and Kuchroo, 2015), we conclude that IL-17A has a more dominant role in driving pathological changes in psoriatic skin than IL-17F.

To identify *in vivo* IL-17A responsive proteins, we treated psoriasis patients with a single dose of secukinumab, a selective anti-IL-17A antibody whose pharmacology and clinical efficacy in psoriasis has been previously demonstrated [11, 12, 23]. Secukinumab has been shown in the patients evaluated in this study to reach pharmacologically active skin concentrations at the time of sampling [19]. Proteomics analysis after selective blockade with secukinumab showed that most changes occurred in the dermis. Many of the most strongly down-modulated proteins mapped to the canonical IL-17A pathway (MetaCore from Thomson Reuters) and were linked to keratinocyte activation, inflammation and neutrophil and leukocyte/dendritic cell recruitment [24]. Three out of the 10 top downregulated proteins (MPO, CXCL1, CXCL5) were neutrophil associated; verifying previously described effects of anti-IL17A therapy on this hallmark feature of psoriasis [25]

and the established role of IL-17A in inducing neutrophil recruitment. As previously seen with transcriptional analysis, the protein expression of IL-1β, MMP-1 and MMP-8 in skin decreased after IL-17A blockade. However, the changes were limited to the skin and not seen in the serum, likely due to the restricted local production and action of IL-1β and MMPs. Similarly, the chemokines CXCL1, CXCL3, CXCL5, CCL1 and CCL20 were also decreased in the skin but not serum after secukinumab, destroying the chemokine gradient from serum into skin. While TNFα mRNA is decreased after secukinumab[12], we did not detect changes in protein levels in this study. The two proteins that increased most in dISF were IgE and CCL24, suggesting a skewing of the skin immune response from Th17 towards Th2. Interestingly, mRNA for IL-17E (IL-25), which promotes Th2 was also slightly increased in skin after secukinumab therapy. Thus, IL-17A blockade leads to decreases in skin proinflammatory cytokines, decreased destruction of the connective tissues, a skewing of the immune response away from Th17, and decreased recruitment of leukocytes particularly neutrophils into the skin.

BD-2 was the protein with by far the biggest change in the dermis and serum following anti-IL-17A treatment (Figure 2 and Table 1). This confirms on a protein level the finding that levels of *DEFB4A* mRNA encoding BD-2 protein are decreased after IL-17A blockade [12, 13]. *DEFB4A* mRNA is highly induced by IL-17A in epidermal keratinocytes and reconstituted human epidermis [13, 26-28] and BD-2 protein is primarily located in the epidermal layer of the skin [29, 30] where it is a potent antimicrobial peptide [31]**.** In addition, BD-2 is chemotactic for cells which express CCR2 [32] and/or CCR6 [33], such as neutrophils [32, 34], monocytes/macrophages [32], immature dendritic cells and T-cells [35], and is involved in the arrest of Th17 cells on inflamed endothelium [27]. Our study confirms BD-2 is expressed in skin by epidermal keratinocytes, and recent results that its expression is highly induced by IL-17A in a synergistic manner with TNFα [36]. Thus, BD-2 is an IL-17A responsive innate inflammatory protein produced in the skin by epidermal keratinocytes.

With the above information, we sought to define if BD-2 could be validated as a biomarker of IL-17A mediated skin pathology in psoriasis. We also recognized that a serum biomarker would be more useful than one that would require a skin biopsy or

microperfusion. Previously, Jansen and colleagues showed that serum BD-2 levels correlated with Pso disease severity as defined by PASI [29]. We have confirmed this finding with a larger cohort of psoriasis patients, and have extended it by showing that serum BD-2 levels correlate even more strongly with IL-17A. Importantly, neutralizing IL-17A with secukinumab decreased BD-2 levels not only in skin, but also in serum, and this reduction occurred within 1 week – the earliest time point that we measured. Given the dramatic reduction seen at 1 week post secukinumab treatment it is likely that BD-2 responds even earlier. Lastly, we tested BD-2 levels in baseline serum samples from patients with diseases thought to be IL-17A driven, and showed that BD-2 levels were most highly elevated in patients with psoriatic skin inflammation. However, there are caveats and limitations of this study. First, many of the mechanistic findings in the dermis were made in a relatively small number of subjects. However, we could confirm a link between the dermis and serum in our cohort and then reproduce many findings in serum from a larger cohort. To confirm the dermal rather than serum data, larger studies using the skin microdialysis would be needed. By IHC, other candidates for soluble biomarkers of IL-17A activity in psoriasis have emerged including S100A7 and S100A8 [13], but these proteins were not evaluated here. BD-2 may also respond to mediators other than IL-17A, and our study shows that IL-17A acts in synergy with TNFα to induce BD-2 by skin epithelium. There are conflicting data regarding the effect of TNFα blockade or other effective psoriasis treatments on BD-2 levels. In one study, *DEFB4A* mRNA levels in skin biopsies did not change after etanercept while they did decrease dramatically with IL-17A blockade [13]. In another study, BD-2 protein staining was qualitatively decreased by IHC after etanercept [15]. There was no decrease, and possibly an increase, of serum BD-2 after treatment with fumaric acid esters [37]. Increased serum BD-2 has been linked to atopic dermatitis and systemic lupus erythematosus [38-40], and BD-2 can be expressed by other epithelia than skin, including lung and gastrointestinal [41, 42]. While we did not detect high BD-2 levels in gut inflammation (CD), we have not tested lung diseases. BD-2 is not an exclusive biomarker for IL-17A mediated skin pathology in psoriasis. However, all studies (including the current one) testing the association of serum or skin BD-2 protein, and/or

skin *DEFB4A* mRNA with psoriasis disease activity, serum IL-17A, or response to IL-17A blockade are consistent and robust.

In summary, our study shows that IL-17A is a dominant driver of skin pathology in psoriasis and serum BD-2 is an easily measurable biomarker of psoriasis disease activity that responds rapidly and robustly to IL-17A inhibition by secukinumab. Serum BD-2 may be a surrogate for IL-17A activity, and could be used to monitor responses to IL-17A-targeted therapies.

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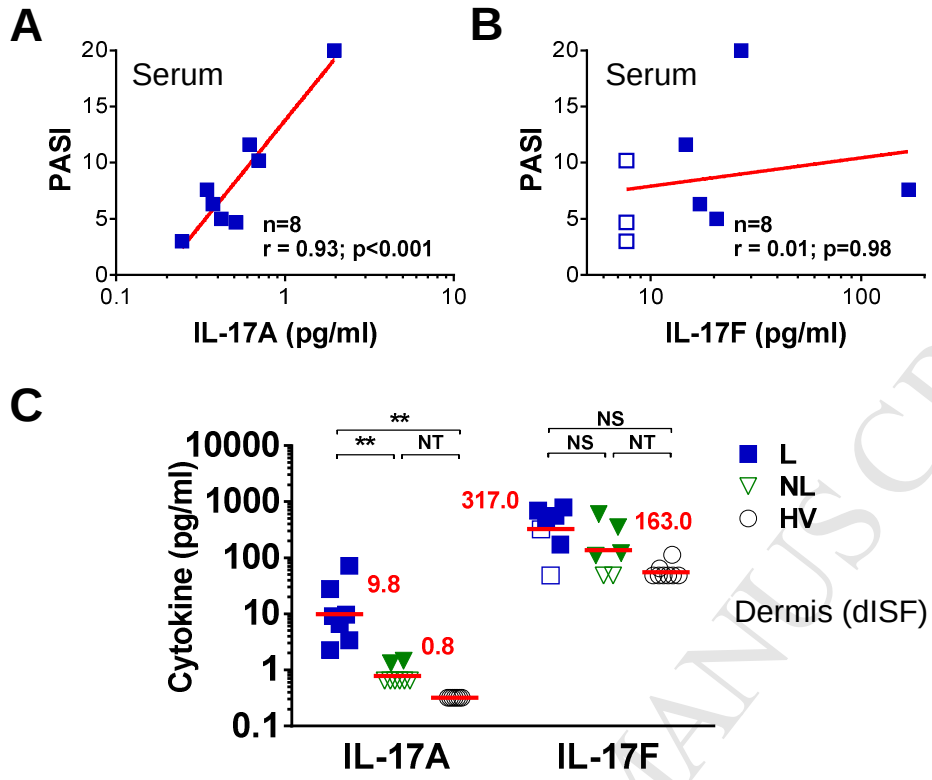


Figure 1: Baseline dermal protein levels of IL-17A are significantly increased in lesional versus non-lesional psoriasis skin and correlate with disease activity (PASI). Correlation plots of PASI vs IL-17A (A) and of PASI vs IL-17F (B). A linear regression fit is depicted by a red line, the strength of the linear relationship is indicated using the Pearson correlation coefficient r, n indicates the number of values on each plot and the p-value shows the probability that the slope of the true relationship is zero. (C) Baseline IL-17A and IL-17F levels in the dermis (dISF) of healthy volunteers (HV, circles), and lesional (L, squares) and non-lesional (NL, triangles) skin from patients with psoriasis. Red lines and values represent the Adjusted Geometric Means and are adjusted for relative recovery of the reference substance, sinistrin (Dragatin et al 2015). Data below LLOQ were imputed as half LLOQ and are shown as open symbols. ***p<0.001; **p<0.01; NS= not significant (p>0.05); NT = Not testable due to number of samples below LLOQ in both groups.

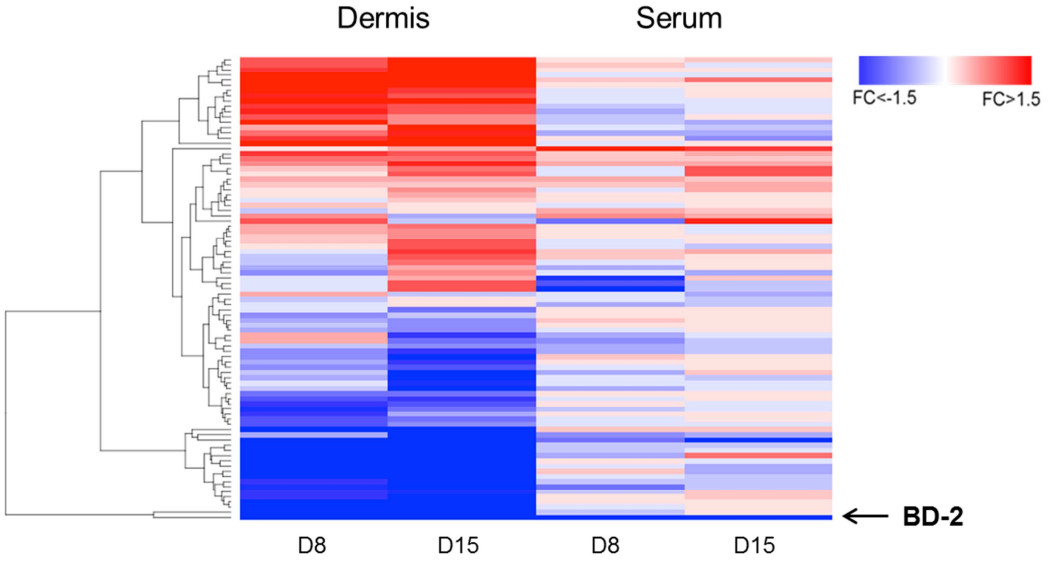


Figure 2. Proteomic analysis of dermis (dISF) and serum from psoriasis patients after IL-17A blockade reveal early molecular changes and identify BD-2 as a potential IL-17A response biomarker. Heatmap of 89 proteins with at least a 1.5 fold change (FC) at either Day 8 or Day 15 compared to baseline in the dermis (dISF) or circulation (serum) of psoriatic subjects treated at Day 1 with secukinumab. Dermal samples were taken from lesional skin. Hierarchical clustering was performed on the protein concentration data to show patterns of expression of related proteins in different compartments at two time points. Blue represents down-regulation from baseline and red indicates up-regulation.

Table 1. Proteins with the greatest fold change and selected proteins of interest in skin (dermis) or circulation (serum) of subjects with psoriasis following treatment with a single dose of the IL-17A inhibitor secukinumab.

Protein	Fold change relative to baseline			
	Dermis (dISF)		Serum	
	Day 8	Day 15	Day 8	Day 15
Top 10 downregulated				
BD-2	-18.73	-32.20	-3.95	-3.66
MMP-1	-6.20	-15.19	-1.11	1.04
IL-1β	-2.71	-5.47	1.14	1.14
IL-1ra	-2.19	-4.37	-1.47	-2.32
MMP-8	-1.91	-3.42	-1.16	-1.07
MPO	-1.18	-3.20	-1.27	-1.18
GRO-α (*CXCL1*)	-2.63	-3.13	-1.08	-1.17
NGAL (*LCN2*)	-2.14	-2.98	-1.11	-1.12
MIP3-α (*CCL20*)	-2.62	-2.64	-1.24	1.45
ENA-78 (*CXCL5*)	-3.00	-2.50	1.05	-1.02
Other proteins of interest				
GRO-γ (CXCL3)	-1.61	-2.20	-1.16	-1.08
I-309 (CCL1)	-1.34	-1.88	1.09	1.03
TNFα	1.00	1.18	1.04	1.03
Top 5 upregulated				
Endoglin	2.51	2.52	1.04	1.08
Leptin	2.59	2.62	1.09	1.39
Acrp30	1.50	2.72	1.13	-1.04
Eotaxin2	1.56	2.77	1.06	1.14
IgE	1.92	3.19	-1.00	-1.06

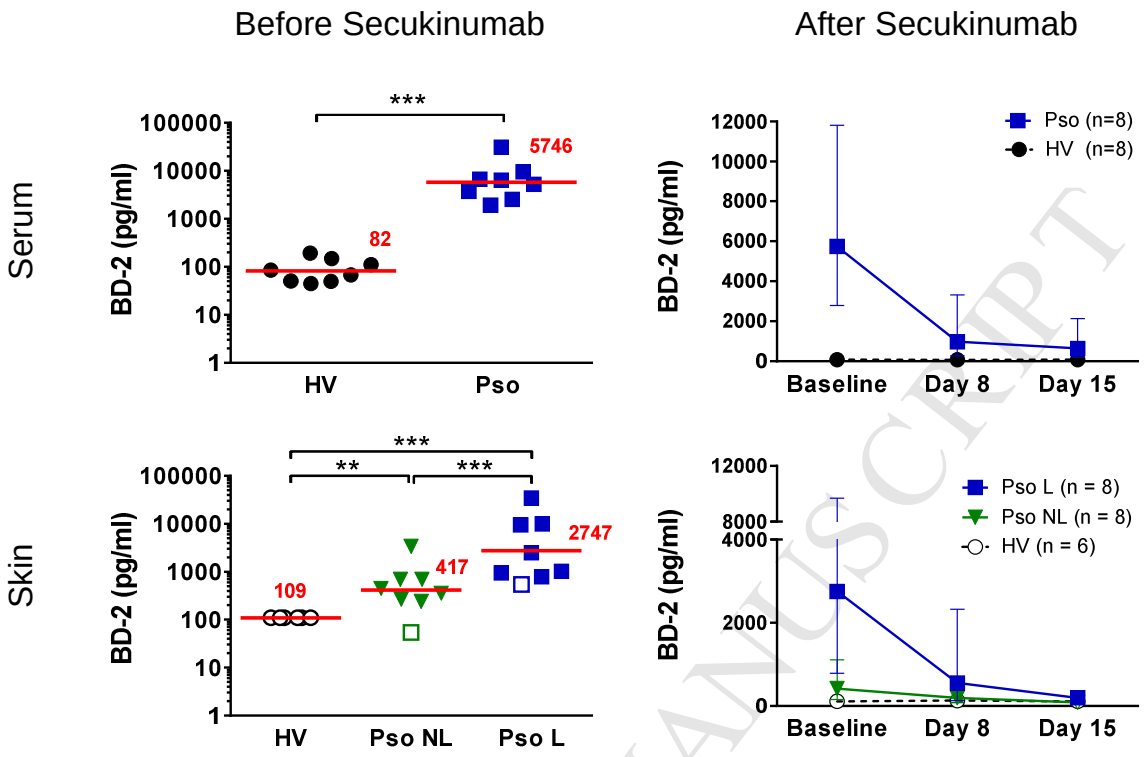


Figure 3: BD-2 protein levels are elevated in psoriatic subjects and rapidly decrease in dermis and serum after secukinumab treatment.

(Upper panel, left) baseline human BD-2 levels in the serum of healthy (HV) and psoriatic subjects (Pso); (Upper panel, right) serum BD-2 levels following a single dose of Secukinumab 300 mg s.c. (Geometric Mean ± 95% CI); (Lower panel, left) baseline BD-2 levels in the dermis (dISF) of lesional (Pso L) and non-lesional (Pso NL) skin from psoriatic subjects and skin from healthy subjects (HV); and (Lower panel, right) dermal BD-2 levels following a single dose of Secukinumab 300 mg s.c. (Geometric Mean ± 95% CI). Red horizontal bars depicts the Geometric Mean concentration. All data below the LLOQ (218pg/ml for HV; 109pg/ml for Pso) were imputed as half LLOQ and are shown as open symbols. ***p<0.001; **p<0.01.

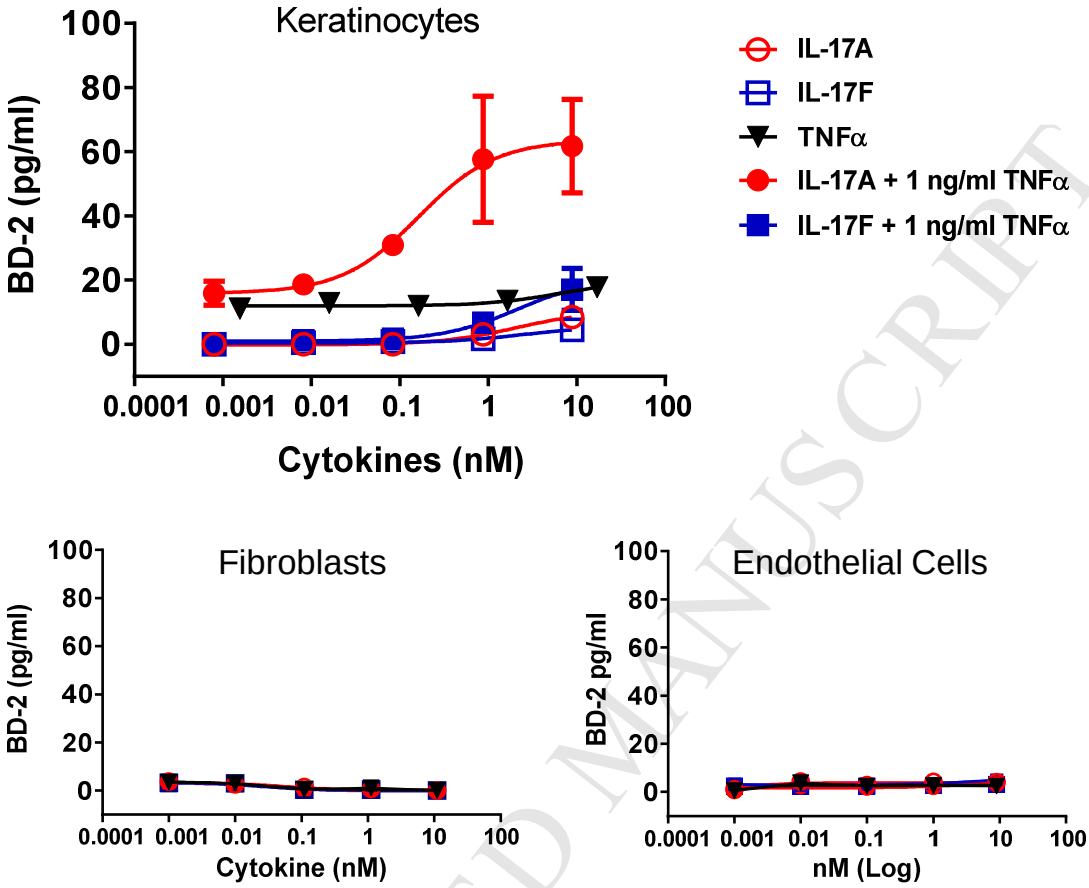


Figure 4: BD-2 expression by different primary human skin cell types. Levels of BD-2 (mean±SD, n=3) in supernatants of either epidermal keratinocytes, dermal fibroblasts or dermal microvascular endothelial cells stimulated with different concentrations of human IL-17A, IL-17F or TNFα alone or IL-17A and IL-17F in combination with a low concentration of TNFα.

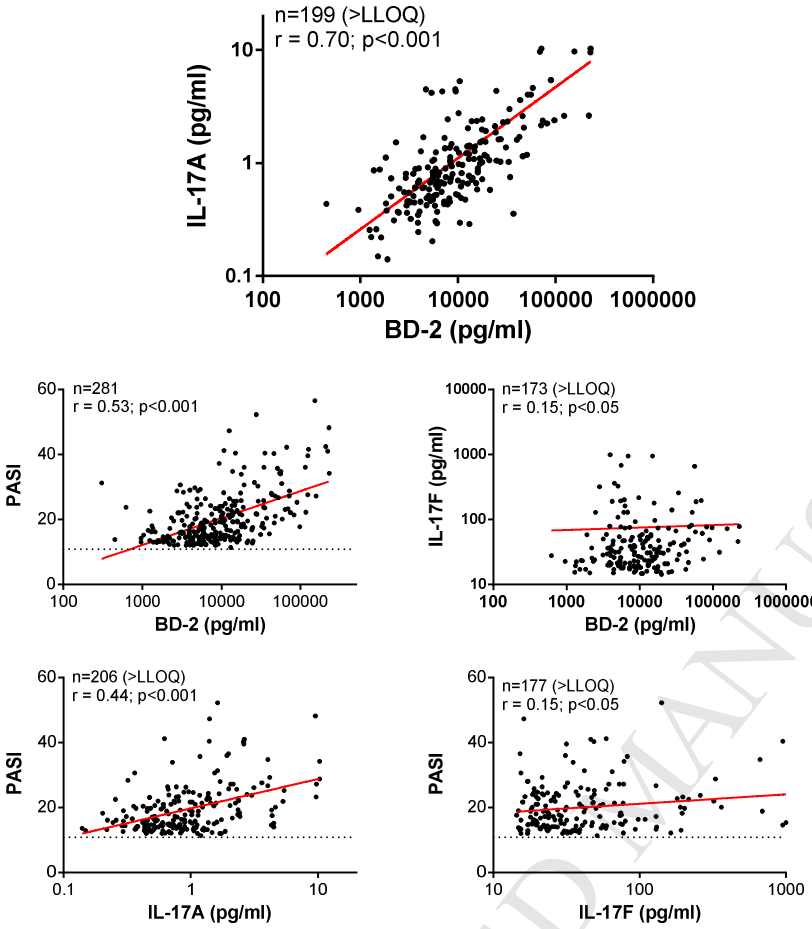


Figure 5: Baseline serum BD-2 levels correlate with IL-17A levels and disease activity (PASI). Correlation plots of PASI and protein levels from stored samples from the AIN457 Regimen Finding Study in Patients with Moderate to Severe Psoriasis (ClinicalTrials.gov Identifier: NCT00941031 data): (Upper panel) IL-17A vs BD-2; (Middle panel, left) PASI vs. BD-2; (Middle panel, right) IL-17F vs. BD-2; (Lower panel, left) PASI vs. IL-17A; and (Lower panel, right) PASI vs IL-17F. In each plot the red line is from a linear regression fit, the strength of the linear relationship is indicated using the Pearson correlation coefficient r; n indicates the number of values on each plot and the p-value is from a test that the slope of each linear regression line equal zero, the smaller the p-value the less likely it is that the slope of the true relationship equals zero.

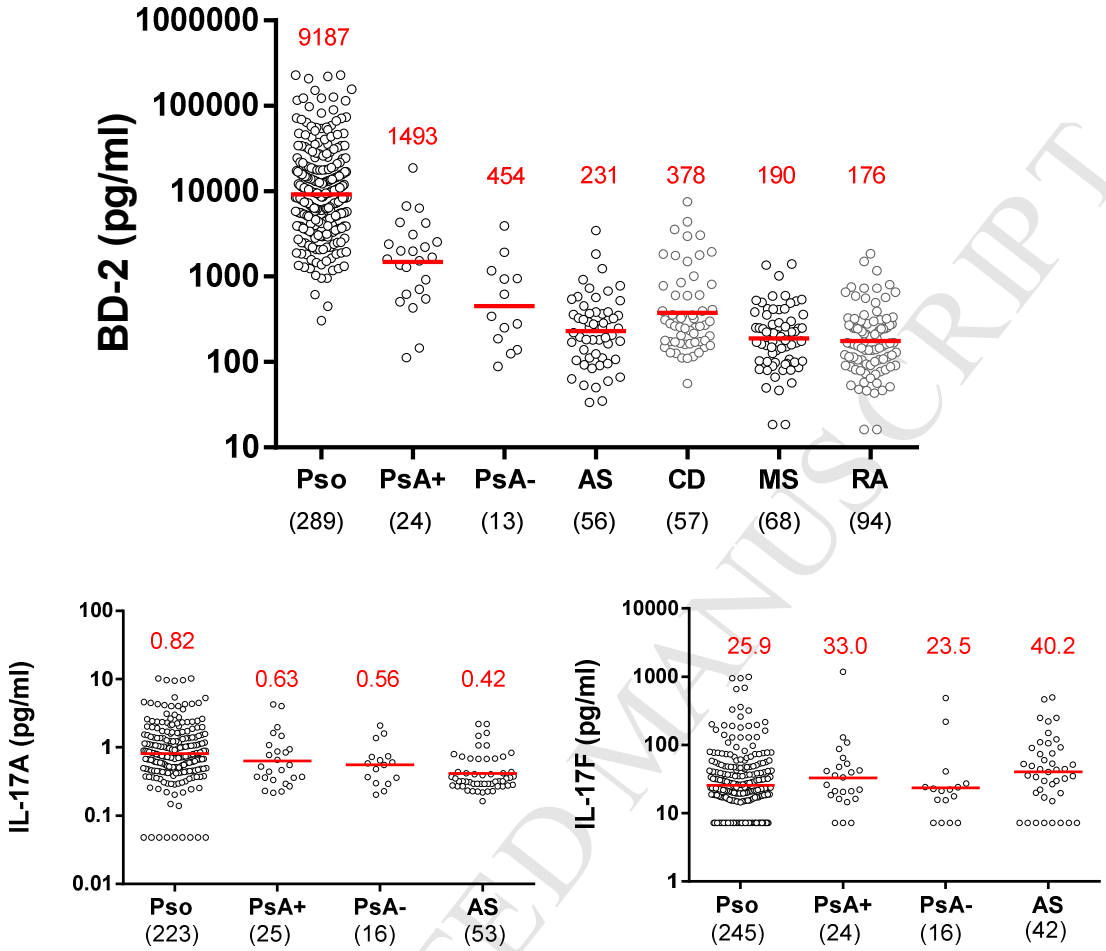


Figure 6: Baseline BD-2, IL-17A and IL-17F serum levels in autoimmune diseases (log scale). Distribution of BD-2, IL-17A and IL-17F baseline levels across indications from 6 different clinical studies. The number in parentheses denotes the number of samples tested. The red line indicates the location of each geometric mean value and its value is indicated by the red text.

Figure S1: Expression of IL-17 family member genes in skin biopsies from subjects with psoriasis and healthy subjects before and after treatment with secukinumab

Quantitative RT polymerase chain reaction (qRT-PCR) was performed on skin biopsies from lesional skin of subjects with psoriasis enrolled in the study (n=6[1]). Healthy control skin biopsies (n = 10) were obtained from a commercial source (Asterand). *Y*-axes show normalized relative quantities (NRQ). Red lines depict median values. **p<0.01; *p<0.05; NS= not significant (p>0.05). [1]Data from 2 subjects not shown due to missing data at either BL or D8.

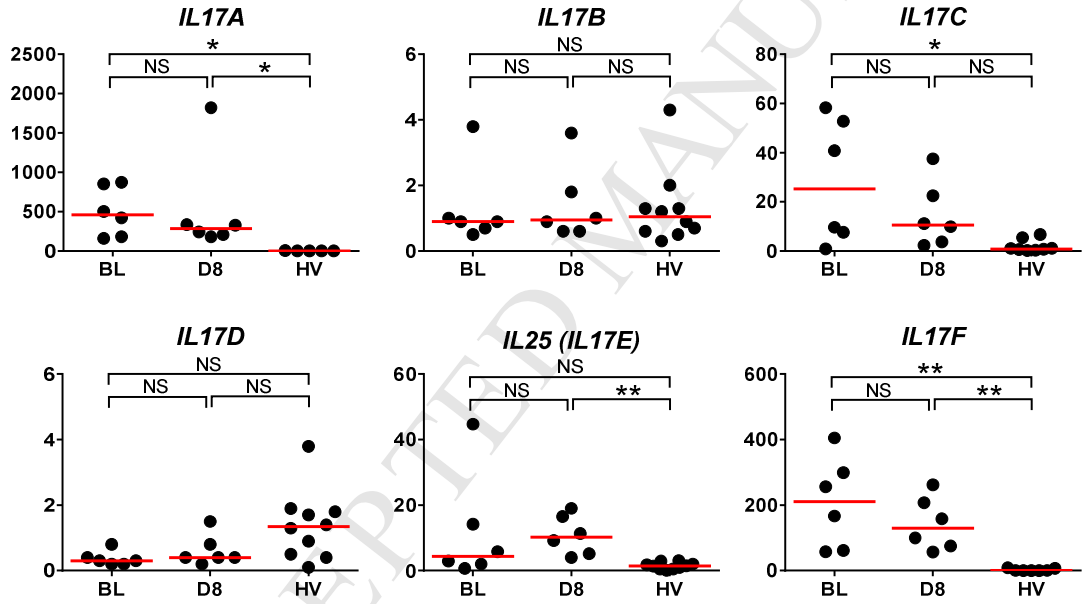


Figure S2: Early transcriptional downregulation of β-defensins and antimicrobial peptides.

mRNA gene expression from lesional skin biopsies and healthy subjects were all determined by Nanostring. Healthy control skin biopsies (n = 10) were obtained from a commercial source (Asterand). Y-axes show normalized relative quantities (NRQ). Red lines depict median values. **p<0.01; *p<0.05; NS= not significant (p>0.05).

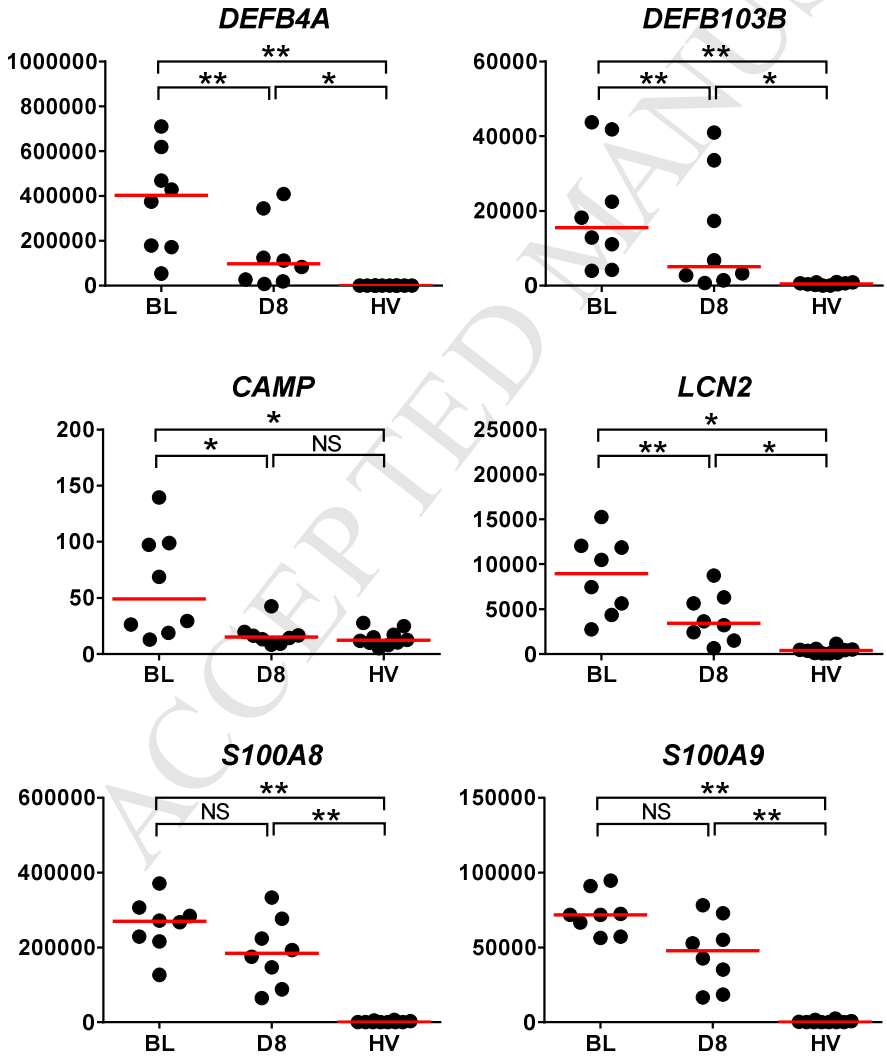


Figure S3: mRNA expression analysis indicates early effects on Th17 and neutrophil attracting chemokines.

mRNA gene expression from lesional skin biopsies and healthy subjects were all determined by Nanostring. Healthy control skin biopsies (n = 10) were obtained from a commercial source (Asterand). Y-axes show normalized relative quantities (NRQ). Red lines depict median values. **p<0.01; *p<0.05; NS= not significant (p>0.05).

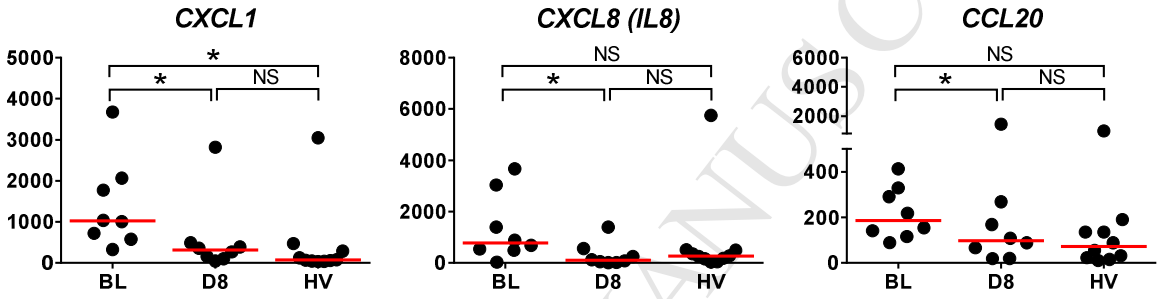
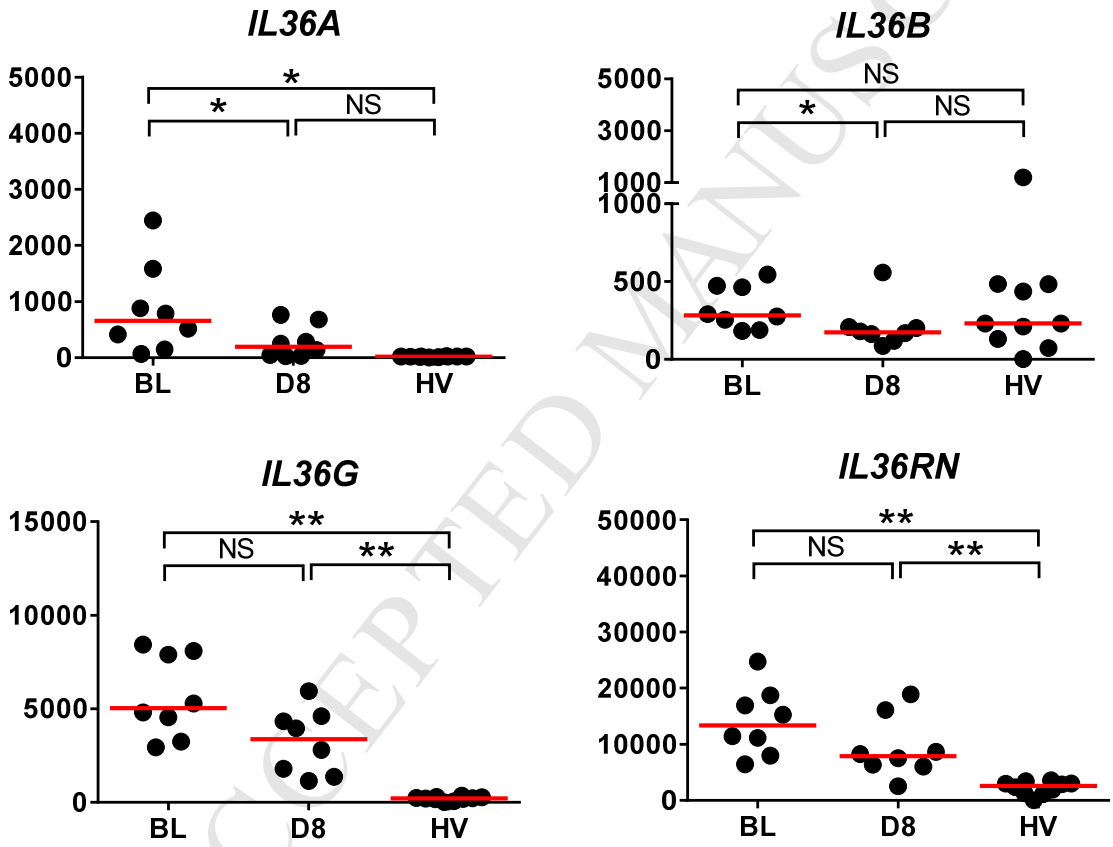


Figure S4: mRNA expression analysis indicates early effects of IL-17A blockade on IL-1 family cytokines IL-36α, IL-36β, and IL-36γ.

mRNA gene expression from lesional skin biopsies and healthy subjects were all determined by Nanostring. Healthy control skin biopsies (n = 10) were obtained from a commercial source (Asterand). Y-axes show normalized relative quantities (NRQ). Red lines depict median values. **p<0.01; *p<0.05; NS= not significant (p>0.05).



Supplementary Table 1: Aushon protein analysis panel

Protein	Entrez Gene ID
α2-Macroglobulin	2
Acrp-30 (Adiponectin)	9370
Ang-2 (Angiopoietin 2)	285
Apo A-1 (Apolipoprotein A-1)	335
Apo B-100 (Apolipoprotein B-100)	338
AR (Amphiregulin)	374
β-NGF (Beta Nerve Growth Factor)	4803
sAPPβ (soluble Amyloid Precursor Protein beta)	351
BCA-1/CXCL13 (B Cell-Attracting Chemokine)	10563
BD-2 (Beta Defensin 2)	1673
BDNF (Brain-Derived Neurotrophic Factor)	627
BMP-9 (Bone Morphogenetic Protein 9)	2658
Cathespin-D	1509
CC16 (Clara Cell Protein)	7356
CD14 (Cluster of Differentiation 14)	929
CD30/TNFRSF8 (Cluster of Differentiation 30)	943
CD40L (Cluster of Differentiation 40 Ligand)	959
CGα (Chorionic Gonadotropin alpha)	1081
Clusterin	1191
CNTF (Ciliary Neurotrophic Factor)	1270
COX-2 (Cyclooxygenase 2)	5743
C-peptide	3630
CRP (C-reactive protein)	1401
E-Cadherin	999
EGF (Epidermal Growth Factor)	1950
EGFR (Epidermal Growth Factor Receptor)	1956
ENA-78 (Epithelial Cell-Derived Neutophil-Activating Peptide 78)	6374
Endoglin	2022
Eotaxin	6356
Eotaxin-2	6369
Eotaxin-3	10344
ER (Epiregulin)	2069
ErbB2/Her2 (Human Epidermal Growth Factor Receptor 2)	2064
E-Selectin	6401
Exodus-2 (6Ckine)	6366
FasL (Fas Ligand)	356
FGF basic (Fibroblast Growth Factor basic)	2247
Fibrinogen	2243
Fibronectin	2335
G-CSF (Granulocyte Colony-Stimulating Factor)	1440
GDNF (Glial Cell-Derived Neurotrophic Factor)	2668
GM-CSF (Granulocyte Macrophage Colony-Stimulating Factor)	1437
gp130 (Glycoprotein 130)	3572
GROα (Growth-Regulated Protein alpha)	2919
GROγ (Growth-Regulated Protein gamma)	2921

HCC-4/CCL-16 (Hemofiltrate CC Chemokine 4)	6360
HGF (Hepatocyte Growth Factor)	3082
HGH (Human Growthh Hormone)	4261
I-309	6346
ICAM-1 (Intercellular Adhesion Molecule1)	3383
ICAM-3 (Intercellular Adhesion Molecule 3)	3385
IFNα (Interferon alpha)	3440
IFNγ (Interferon gamma)	3458
IgE (Immunoglobulin E)	
IGFBP-1 (Insulin-like Growth Factor Binding Protein 1)	3484
IGFBP-2 (Insulin-like Growth Factor Binding Protein 2)	3485
IGFBP-3 (Insulin-like Growth Factor Binding Protein 3)	3486
IL-1α (Interleukin 1 alpha)	3552
IL-1β (Interleukin 1 beta)	3553
IL-1ra (Interleukin 1 Receptor Antagonist	3557
IL-1RI (Interleukin 1 Receptor I)	3554
IL-1RII (Interleukin 1 Receptor II)	7850
IL-2 (Interleukin 2)	3558
IL-2Rα (Interleukin 2 Receptor alpha)	3559
IL-2Rγ (Interleukin 2 Receptor gamma)	3561
IL-3 (Interleukin 3)	3562
IL-4 (Interleukin 4)	3565
IL-4R (Interleukin 4 Receptor)	3566
IL-5 (Interleukin 5)	3567
IL-6 (Interleukin 6)	3569
IL-6R (Interleukin 6 Receptor)	3570
IL-7 (Interleukin 7)	3574
IL-8 (Interleukin 8)	3576
IL-9 (Interleukin 9)	3578
IL-10 (Interleukin 10)	3586
IL-11 (Interleukin 11)	3589
IL-12p40 (Interleukin 12 p40 homodimer)	3593
IL-12p70 (Interleukin 12 p70 heterodimer)	3592 and 3593
IL-13 (Interleukin 13)	3596
IL-13Rα1 (Interleukin 13 Receptor alpha 1)	3597
IL-15 (Interleukin 15)	3600
IL-16 (Interleukin 16)	3603
IL-17A (Interleukin 17A)	3605
IL-17E (Interleukin 17E)	64806
IL-18 (Interleukin 18)	3606
Insulin	3630
IP-10 (Interferon gamma-Induced Protein 10 kDa)	3627
I-TAC (Interferon-Inducible T-cell Alpha Chemoattractant)	6373
KGF (Keratinocyte Growth Factor)	2252
Leptin	3952
LIF (Leukemia Inhibitory Factor)	3976
L-Selectin	6402
Lymphotactin	6375

MCP-1 (Monocyte Chemotactic Protein 1)	6347
MCP-2 (Monocyte Chemotactic Protein 2)	6355
MCP-3 (Monocyte Chemotactic Protein 3)	6354
MCP-4 (Monocyte Chemotactic Protein 4)	6357
MDC (Macrophage-Derived Chemokine)	6367
MIF (Migration Inhibitory Factor)	4282
MIG (Monokine Induced by Gamma Interferon)	4283
MIP-1α (Macrophage Inflammatory Protein 1 alpha)	6348
MIP-1β (Macrophage Inflammatory Protein 1 beta)	6351
MIP-1δ (Macrophage Inflammatory Protein 1 delta)	6359
MIP-3α (Macrophage Inflammatory Protein 3 alpha)	6364
MIP-3β (Macrophage Inflammatory Protein 3 beta)	6363
MIP-4/PARC (Macrophage Inflammatory Protein 4, Pulmonary and Activation-Regulated Chemokine)	6362
MMP-1 (Matrix Metalloproteinase 1)	4312
MMP-2 (Matrix Metalloproteinase 2)	4313
MMP-3 (Matrix Metalloproteinase 3)	4314
MMP-7 (Matrix Metalloproteinase 7)	4316
MMP-8 (Matrix Metalloproteinase 8)	4317
MMP-9 (Matrix Metalloproteinase 9)	4318
MMP-10 (Matrix Metalloproteinase 10)	4319
MMP-13 (Matrix Metalloproteinase 13)	4322
MPIF-1 (Myeloid Progenitor Inhibitory Factor 1)	6368
MPO (Myeloperoxidase)	4353
NAP-2 (Neutrophil Activating Peptide 2)	5473
NGAL (Neutrophil Gelatinase-Assocciated Lipocalin)	3934
NT3 (Neurotrophin 3)	4908
NT-proBNP (N-Terminal Prohormone of Brain Natriuretic Peptide)	4879
OPG (Osteoprotegrin)	4982
OPN (Osteopontin)	6696
PAI-1 Active (Plasminogen Activator Inhibitor 1 Active	5054
PAI-1 Total (Plasminogen Activator Inhibitor 1 Total)	5054
PAPP-A (Pregnancy-Associated Plasma Protein A)	5069
PD-1 (Programmed Death 1)	5133
PDGF-AA (Platelet-Derived Growth Factor AA)	5154
PDGF-AB (Platelet-Derived Growth Factor AB)	5154 and 5155
PDGF-BB (Platelet-Derived Growth Factor BB)	5155
PECAM-1 (Platelet Endothelial Cell Adhesion Molecule)	5175
PEDF (Pigment Epithelium-Derived Factor)	5176
PLGF (Placental Growth Factor)	5228
Prolactin	5617
Protein C	5624
P-Selectin	6403
RAGE (Receptor for Advanced Glycation End Products)	177
RANK (Receptor Activator of NF-κB)	8792
RANKL (Receptor Activator of NF-kB Ligand)	8600
RANTES (Regulated upon Activation, Normal T cell Expressed and presumably Secreted)	6352

RBP4 (Retinol Binding Protein 4)	5950
Resistin	56729
SAA (Serum Amyloid A)	6288
SCF (Stem Cell Factor)	4254
SDF-1 (Stromal Cell-Derived Factor 1)	6387
SHBG (Sex Hormone Binding Globulin)	6462
TARC (Thymus and Activation-Regulated Chemokine)	6361
TFF-3 (Trefoil Factor 3)	7033
TGFα (Transforming Growth Factor alpha)	7039
TGFβ1 (Transforming Growth Factor beta 1)	7040
TGFβ2 (Transforming Growth Factor beta 2)	7042
TM (Thrombomodulin)	7056
TIM-1 (T Cell Immunoglobulin Mucin 1)	26762
TIMP-1 (Tissue Inhibitor of Metalloproteinases 1)	7076
TIMP-2 (Tissue Inhibitor of Metalloproteinases 2)	7077
TNFα active trimer (Tumor Necrosis Factor alpha)	7124
TNFα monomer + trimer (Tumor Necrosis Factor alpha)	7124
TNF-RI (Tumor Necrosis Factor alpha Receptor I)	7132
TNF-RII (Tumor Necrosis Factor alpha Receptor II)	7133
TPO (Thrombopoietin)	7066
TRAIL (TNF-Related Apoptosis-Inducing Ligand)	8743
TSLP (Thymic Stromal Lymphopoietin)	85480
TSP-1 (Thrombospondin-1)	7057
TSP-2 (Thrombospondin-2)	7058
TWEAK (TNF-related and WEAK induder of apoptosis)	8742
VCAM-1 (Vascular Cell Adhesion Molecule 1)	7412
VEGF (Vascular Endothelial Growth Factor)	7422
VEGF-C (Vascular Endothelial Growth Factor C)	7424
VEGF-D (Vascular Endothelial Growth Factor D)	2277
VEGF-R1 (Vascular Endothelial Growth Factor Receptor 1)	2321
VEGF-R2 (Vascular Endothelial Growth Factor Receptor 2)	3791

Supplementary Methods: Gene expression analysis

Total RNA extraction. 4 mm punch skin biopsy tissue samples were collected and immersed in 1.5 mL RNAlater® RNA Stabilization Reagent. Total RNA was isolated from tissue samples using the Qiagen RNeasy Micro Kit and eluted in 12 µL RNase-free water. RNA isolated from commercial skin biopsies (Asterand UK Limited) from healthy subjects (n = 10) served as controls for gene expression data. 20-50 ng of RNA was pre-amplified using the Affymetrix SensationPlus™ FFPE Amplification kit.

NanoString nCounter gene expression analysis: RNA samples were processed with the nCounter Prep Station and Digital Analyzer of NanoString Technologies. The custom-designed nCounter Gene Expression CodeSets C1933_Amadeus and C2018_Everest overall contained probe sets for 314 target transcripts, seven candidate reference genes for normalization, and two gender control genes. Probe sequences for genes reported in this study are shown in Supplementary Table 2. 300 ng (C1933_Amadeus) or 25 ng (C2018_Everest) of the pre-amplified sense RNA was hybridized with the respective CodeSet at 65℃ for 16 h. Post-hybridization proces sing procedures were carried out as recommended by NanoString Technologies. Cartridges were scanned at a resolution of 600 fields of view. Gene expression barcode counts were analyzed using the nSolver Analysis software v1.1 (NanoString Technologies). Raw NanoString barcode counts for each gene were subjected to a mRNA content-related normalization using the geometric mean of the three reference genes (*RPL13A*, *RPL19*, and *UBC*).

qRT-PCR gene expression analysis: qRT-PCR was applied for the expression analysis of IL-17 family member genes. However, at the time of qRT-PCR analysis, two individual RNA samples from two different subjects (one from baseline, the other one from day 8,

which had the lowest total RNA yield after extraction) had already been exhausted by preceding microarray and Nanostring gene expression analyses. Complementary DNA (cDNA) synthesis from mRNA was performed using the iScript advanced cDNA synthesis kit (Bio-Rad Laboratories) with 2000 ng input of pre-amplified sense RNA generated as described above. Samples were processed with a cDNA (total RNA equivalent) input of 10 ng/reaction (*RPL13A, RPL19, UBC*) or 40 ng/reaction (*IL17A, IL17B, IL17C, IL17D, IL17E, IL17F*), using pre-designed probe based qPCR assays from Integrated DNA Technologies or Life Technologies (*IL17A*: Hs.PT.56a.19213466; *IL17B*: Hs.PT.58.2931881; *IL17C*: Hs.PT.56a.1093657; *IL17D*: Hs00370528_m1; *IL25* (*IL17E*): Hs03044841_m1; *IL17F*: Hs.PT.56a.20717359; *RPL13A*: Hs.PT.56a.39648480.g; *RPL19*: Hs.PT.56a.39989087; *UBC*: Hs.PT.39a.22214853). All samples were measured in triplicate using a QuantStudio 12K Flex instrument (Applied Biosystems). The Real-Time program was 50℃ for 2 minutes, 95℃ for 10 minutes (1 cycle); 95℃ for 15 seconds; and 60℃ for 60 seconds (40 cycles). Automatic threshold settings were used. qRT-PCR results were evaluated using the QuantStudio 12k Flex software v1.1.1, (Applied Biosystems). For qRT-PCR, normalized relative quantities (NRQs) were calculated according to the method described by Hellemans and colleagues [Hellemans et al 2007]. The geometric mean of the quantification cycle (Cq) values of all Asterand healthy skin reference samples was used as a reference Cq value for the calculation of relative quantities (RQs). RQ values were then normalized using the geometric mean of the RQs of the 3 reference genes, *RPL13A*, *RPL19* and *UBC*. According to the applied calculations a NRQ value of one reflects the geometric mean of the respective gene expression in Asterand healthy skin reference samples.

Supplementary Table 2. Nanostring codeset probe sequences for genes reported in this study

Gene identifier	Capture probe sequence	Reporter probe sequence
CAMP	CTATAGCACGAAGCACAGCTTCCTTGT AGCTGAGGACCTGGGCAATGATG	GTAGAGGTTAGCATCCGAGGACCGCTGG TTGATGCCAT
CCL20	CAGGAGCAAACTCTTGGTACAGCACAT GGTTTTTAGCTCAAAGAACAGAT	GCTTCTGATTCGCCGCAGAGGTGGAGTA GCAGCACTGACATCAAAGCAGC
CXCL1	CTCTATCACAGTGGCTGGCATGTTGCA GGCTCCTCAGAAATATTAACATA	TTCACAATGATCTCATTGGCCATTTGCTT GGATCCGCCAGC
CXCL8 (IL8)	CCGGTGGTTTCTTCCTGGCTCTTGTCCT AGAAGCTTGTGTG	AGCCACGGCCAGCTTGGAAGTCATGTTT ACACACAGTGAGATG GTTCCTT
DEFB4A	TATGGCTCCACTCTTAAGGCAGGTAAC AGGATCGCCTATACCA CCAAAAA	CCACAGGTGCCAATTTGTTTATACCTTCT AGGGCAAAAGACTGG ATGACA
DEFB103B	TTTTTATTTCTTTCTTCGGCAGCATTT TCGGCCACGCGTCGAGC ACTTG	CTTTAAGAAGGCATTTCCACACTTTACA ACACTCTCGTCATGTTT CAGGG
LCN2	CTGATCCAGTAGTCACACTTCTTTTTCC TAAACAGGACGGAGGTGACATT	TAATGTTGCCCAGCGTGAACTCGCCGGGC TGGCAACCTGGAACAAAAGTC
RPL13A	TCCTTGCTCCCAGCTTCCTATGTCCCAG GGCTGCC	ATTCTCCGAGTGCTTTCAAGCAACTTCGG GAGGCAGTGACTAA GACCCTT
RPL19	AATCCTCATTCTCCTCATCCATGTGACC TTCTCTGGCATTCGG GCATTGG	TGGCGATCGATCTTCTTAGATTCACGGTA TCTTCTGAGCAGCC GGCGCAA
UBC	CACTTCGAGAGTGATGGTCTTACCAGT CAGGGTCTTCACGAAG ATCTGCA	TCCTTGTCTTGGATCTTTGCCTTGACATT CTCAATGGTGTCACT CGGCTC