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Characterization of innate lymphoid cells (ILC) in human skin and blood demonstrates increase of NKp44+ ILC3 in psoriasis

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Abstract

Innate lymphoid cells (ILC) are increasingly appreciated as key regulators of tissue immunity. However, their role in human tissue homeostasis and disease remains to be fully elucidated. Here we characterise the ILC in human skin from healthy individuals and from the inflammatory skin disease psoriasis. We show that a substantial proportion of IL-17A and IL-22 producing cells in skin and blood of normal individuals and psoriasis patients are CD3 negative innate lymphocytes. Deep immunophenotyping of human ILC subsets showed a statistically significant increase in the frequency of circulating NKp44+ ILC3 in blood of psoriasis patients compared to healthy individuals or atopic dermatitis patients. More than 50% of circulating NKp44+ ILC3 expressed cutaneous lymphocyte-associated antigen indicating their potential for skin homing. Analysis of skin tissue revealed a significantly increased frequency of total ILC in skin compared to blood. Moreover the frequency of NKp44+ ILC3 was significantly increased in non-lesional psoriatic skin compared to normal skin. A detailed time course of a psoriasis patient treated with anti-TNF showed a close association between therapeutic response, decrease in inflammatory skin lesions, and decrease of circulating NKp44+ ILC3. Overall, data from this initial observational study suggest a potential role for NKp44+ ILC3 in psoriasis pathogenesis.

Introduction

The skin is the primary interface with the external environment and thus it is required to provide the first line of host defence against injury and infection (Di Meglio *et al.*, 2011; Kupper and Fuhlbrigge, 2004). Similar to lung and gut mucosal barrier, the skin is equipped with a diverse set of immune cells that are poised to react to different insults, yet retain the potential to cause autoimmunity.

Plaque-type psoriasis is a chronic inflammatory skin disease characterized by highly inflamed scaly lesions resulting from hyperproliferation of the epidermis and a prominent

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Conflict of interest FN has been a consultant for companies producing anti-TNF reagents for treatment of patients with psoriasis.

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inflammatory infiltrate (Griffiths and Barker, 2007; Nestle *et al.*, 2009). The immunopathogenesis of psoriasis is based on a combination of genetic susceptibility and environmental risk factors triggering a pathogenic cross-talk between innate and adaptive immune cells (Lowes *et al.*, 2013). Recent advances in understanding the genetic basis of the disease suggest a significant involvement of the innate immune system. Indeed, 11 out of 36 psoriasis susceptibility loci identified in individuals of European ancestry, encode plausible regulators of innate host defense (Tsoi *et al.*, 2012). The IL-23/IL-17/IL-22 axis bridges innate and adaptive immunity and is of critical importance in psoriasis (Di Cesare *et al.*, 2009; Diveu *et al.*, 2008). While a contribution of conventional αβ T cells to IL-17 and IL-22 cytokine production is well established (Hijnen *et al.*, 2013), the contribution of innate immune cell subsets is less well understood. Recent data in both mouse experimental models, (Pantelyushin *et al.*, 2012) and in human immune-mediated pathologies, such as Crohn's disease (Geremia *et al.*, 2011) have established a potential role of innate lymphoid cells (ILC) as key sources of IL-17 and IL-22 production in epithelial inflammatory disease.

ILC are recombination activating gene (RAG)-independent cells and are identified by their lymphoid morphology as well as the absence of lineage markers for T/B cells and for other innate cells (Spits *et al.*, 2013). Three ILC groups have been described in both mouse and humans, related by their dependence on the transcriptional repressor Inhibitor of DNA binding 2 (Id2) and on the IL-2Rγ chain (Yokota *et al.*, 1999). The ILC3 subset, which also includes lymphoid tissue inducer cells (LTi), are dependent on the transcription factor RORγT, as well as expression of the IL-7Rα chain, and can produce IL-17A and/or IL-22 upon stimulation (Takatori *et al.*, 2009). In humans ILC3 can be subdivided on the basis of the expression of the natural cytotoxicity receptors (NCRs) NKp44, NKp46 and NKp30 (Cella *et al.*, 2009). $NKp44^+$ ILC can produce IL-22 and are dependent on the Aryl hydrocarbon Receptor (AhR), conversely, human LTi and other NKp44− ILC3 produce IL-17A following stimulation (Hoorweg *et al.*, 2012). However, $NKp44^+$ ILC3 isolated from the tonsil produce both IL-17 and IL-22, while NKp44− ILC3 show considerable plasticity, being able to develop either into NKp44+ ILC3 or into ILC1 cells producing IFNγ (Bernink *et al.*, 2013). ILC3 have been shown to produce IL-17 in the gut of inflammatory bowel disease patients (Bernink *et al.*, 2013), suggesting a potential role in immune-mediated diseases such as colitis and psoriasis.

Despite the growing interest about innate sources of pro-inflammatory cytokines, the ILC populations of human skin are ill-defined. Here, we explore their presence in psoriasis, as well as healthy skin and another inflammatory skin disease, atopic dermatitis (AD). We demonstrate that CD3 negative innate lymphocytes are major contributors to IL-17 and IL-22 production in both blood and skin of healthy individuals and psoriasis patients. We further show that the NKp44+ ILC3 subset of innate lymphoid cells, previously demonstrated to produce IL-17 and IL-22 (Bernink *et al.*, 2013), is increased in frequency in both peripheral blood and skin of psoriasis patients. Interestingly, in a psoriasis patient with a high baseline frequency of NKp44+ ILC3 the clinical response to anti-TNF therapy was associated with a decrease of this population in the blood implying that it may be a either a useful biomarker, or even a contributor to disease.

Results and Discussion

Identification of a population of IL-17 and IL-22 producing CD3 negative immune cells in blood and skin of psoriatic patients

We recently embarked on a systematic analysis of peripheral immune cells in patients with inflammatory skin disease. During our analysis of Th17 cells in the blood of psoriasis patients we observed that there was a consistent population of CD3 negative (CD3−) innate lymphocytes capable of producing IL-17A. Similarly, when we assessed IL-22 production

we could readily identify CD3− cells, which were making IL-22 (Fig. 1a-b). In peripheral blood these cells account for about 20% of IL-17 (normal, mean: 15.65%±1.95; psoriasis, mean: 21.83±4.40%) and about 40% of IL-22 (normal, mean: 41.49%±4.04; psoriasis, mean: 39.95%±4.91) producing cells. To assess whether these cytokine producing CD3− cells are also present in the skin we isolated cells from the skin of normal healthy individuals (N) or patients with either psoriasis (P) or AD (A). Distinct populations of CD3+ and CD3− cells could be clearly identified in the skin and CD3 discrimination was not affected by enzymatic tissue digestion; Fluorescence minus one controls and isotype controls were used to define the CD3 gate and exclude non-specific binding (Fig. S1). In agreement with our peripheral blood data, CD3− IL17+ cells could be clearly identified in the dermis and epidermis of healthy normal skin (NN), lesional (PP) and non-lesional (PN) skin of psoriatic, as well as in lesional (AA) and non-lesional (AN) skin of AD patients (Fig. 1c and S2). CD3− IL-22+ cells could be identified in epidermal samples from healthy volunteers, psoriasis and AD patients; they could not be identified in normal dermis (NN), but could be found in both non-lesional (PN and AN) and lesional samples (PP and AA) from patients with skin disease (Fig. 1 and Fig. S2). The frequency of these cells varied considerably between individuals (Fig. S2) and as a result no statistically significant differences were observed; however, nonlesional psoriatic epidermis seemed to harbour the highest median frequency of CD3− IL-17+ and CD3− IL-22+ cells (15.9 and 3.8%, respectively). Additionally, we performed dermal explant cultures of NN (n=2), PP (n=5) and PN (n=2) skin, allowing cells to migrate out of the tissue over 4-5 days. Migratory immune cells confirmed the presence of CD3− cells capable of producing IL-17 (Fig.1d), as well as IL-22 (data not shown).

Our data point to the existence of an IL-17 and IL-22 producing CD3− immune cell subset in blood and skin of healthy individuals and patients with inflammatory skin disease.

Extensive immunophenotyping of psoriasis peripheral blood cells reveals an enrichment in NKp44+ innate lymphoid cells 3 (ILC3)

To further dissect immune cells subsets in healthy individuals and patients with psoriasis or AD, we performed extensive immunophenotyping of PBMCs. No apparent differences were found in the populations of T, B, NK and myeloid cells between healthy individuals and patients with inflammatory skin disease (Fig. 2a). Based on the recent demonstration of ILCs as important contributors to IL-17 and IL-22 production in models of inflammatory skin disease (Pantelyushin *et al.*, 2012) and gut inflammation (Geremia *et al.*, 2011), we developed a panel of antibodies (Suppl. Table 4) allowing for the differentiation between the major human ILC subsets (Spits *et al.*, 2013). Analysis of the total ILC population defined as lymphoid/CD45+/Lin−/CD127+ in PBMC of healthy individuals showed a mean frequency of 0.06±0.01% (n=9) in agreement with previously reported data (Bernink *et al.*, 2013). The total ILC frequency was similar in psoriasis patients (n=11, mean 0.05±0.01%) compared with healthy controls, but was somewhat decreased in AD patients (n=10, mean 0.03±0.01% $p<0.05$ vs normal) (Fig. 2b). The distinct populations of ILCs were defined as shown in Figure 2b: ILC2 (CRTH2+, green frame), ILC3 (CRTH2− CD117+) and either NKp44− (purple frame) or NKp44+ (orange frame), and ILC1 (CRTH2−/CD117−/CD161+, pink frame). Figure 2c shows the distribution of the different subsets, as mean frequency, in normal, psoriasis and AD patients. In healthy individuals the NKp44− ILC3 population was the most frequent accounting for more than 40% of all ILC (median: 45%, [36-54%]). The frequency of ILC1 and ILC2 varied somewhat among healthy individuals (ILC1 median: 24%, [19-42%] and ILC2 median: 25%, [19-45%])(Fig. 2c). Noticeably, the NKp44+ population of ILC3 was negligible in healthy individuals ranging from undetectable to 1% in 8 out of 9 individuals and 4% in one additional individual. No significant differences were observed in the distribution of ILC subsets in AD patients compared to healthy volunteers

(Fig. 2c). However, in psoriasis patients there was a statistically significant ($p<0.05$) increase in the median frequency of NKp44+ ILC3 cells from 0.2% [0-3.9%] in healthy individuals to 2.6% [0.1-53%] in patients (Fig. 2d). The frequency of NKp44+ ILC3 in psoriasis patients appeared to be bi-modally distributed, with one group of patients having low frequencies similar to those seen in healthy individuals (6 of 11) and a second group in which the NKp44+ ILC3 population ranged from 9.2% to 53% of ILC (5 of 11). Whilst the relatively small number of patients makes it difficult to perform proper stratification, no association was found between the bimodal frequency of NKp44+ ILC3 and either psoriasis patient clinical and demographic features (age, sex , PASI, age of disease onset, presence of co-morbidities), and the frequency of several peripheral cell population (CLA+ ILCs, CD3, CD4 and CD8 T cells, Treg, Th17, NK, monocyte and DC). Further studies are required to confirm or rule out a genetic influence on the cell frequency of this population.

Successful treatment of psoriasis with anti-TNF antibody is associated with a decrease of circulating NKp44+ ILC3: a case study

We next asked whether NKp44+ ILC3 frequencies change during treatment of psoriasis with cytokine targeted therapy. TNF synergizes with IL-23 to drive IL-17A production by ILC (Powell *et al.*, 2012). We had the opportunity to closely follow the clinical progression of patient P21 who had the highest frequency of circulatory NKp44+ ILC3 during treatment with an anti-TNF monoclonal antibody (adalimumab). P21 was off any systemic treatment at the time the initial blood sample was taken and was then commenced on adalimumab. The Psoriasis Area Severity Index (PASI), which measures disease severity, was 21.2 indicating substantial disease. Subsequent blood samples were taken at 1wk, 4wks and 12wks after commencing treatment. There were no gross changes in the frequencies of B cells, NK cells, myeloid cells or T cells during adalimumab therapy (Fig. 3a). In contrast there was a dramatic shift in the frequencies of ILC3 populations in the blood with a 75% reduction in the NKp44+ ILC3 population and a corresponding increase in the NKp44− ILC3 population (Fig. 3b). The shift in the absolute number of NKp44+ ILC3 was closely associated with a reduction in disease severity and extent measured by PASI, which decreased from 21.2 to 13.6 over time during treatment with adalimumab (Fig. 3c).

Although only a single case, this result is potentially instructive; suggesting that frequency of circulating NKp44+ ILC3 might reflect disease severity and/or response to treatment with anti-TNF therapy. Additionally, it highlights a potential role for TNF in the differentiation of human NKp44+ ILC3, which could be the subject of further investigation beyond the scope of this initial descriptive study.

ILC3 preferentially express the skin homing cutaneous lymphoid-associated antigen (CLA)

In order to assess if there is a population of ILC with skin homing potential, we analysed the expression of the skin homing marker CLA on ILC populations and T cells in the blood. Interestingly, ILC of healthy individuals expressed CLA at high frequency (mean 33±4%); significantly higher than on CD8 T cells (mean 11±5%, $p<0.01$) and higher than on CD4 T cells (mean 18±5%) although this did not reach statistical significance (Fig. 4a). A significant increase in the frequency of CLA+ ILC was also seen in the blood of psoriasis and AD patients where the frequency of CLA expression was 2-4 fold higher on ILC than on either CD4 or CD8 T cells (Fig 4a). In healthy individuals the expression of CLA on NKp44− ILC3 was particularly high (mean 48±15%), and significantly increased when compared to ILC2 $p<0.05$ (mean 18±12%). Similarly expression of CLA was also high on NKp44− ILC3 in patients with inflammatory skin disease (psoriasis mean 56±22%; AD mean 56±16%) (Fig. 4b).

NKp44+ ILC3 were only present at high enough frequency in psoriasis patients to assess CLA expression, interestingly these cells expressed similarly high levels of CLA to NKp44− ILC3 and significantly more than ILC2 (Psoriasis mean % CLA+: NKp44+ ILC3 54±18% vs ILC2 14±4%, $p<0.001$). These results imply that both circulating NKp44− and NKp44+ ILC3 express skin homing markers and are poised to home to skin.

NKp44+ ILC3 are increased in non-lesional skin and lesional skin of psoriasis patients

To confirm their role in skin inflammation we isolated and characterized ILC in skin biopsies from psoriasis patients and skin obtained from healthy individuals after surgical procedures. We did not have the opportunity to investigate skin of AD patients where clearly ILC2 are increased in lesional skin (Kim *et al.*, 2013). In preliminary experiments we found that optimal cell yield/viability was obtained by collagenase digestion of whole skin. Gating strategy and representative plots of the different ILC subsets in healthy and psoriasis skin are shown in Figure 4c. ILC populations were significantly enriched in skin compared to blood (PP skin vs P blood $p<0.01$; NN skin vs N blood $P<0.001$), with a median frequency of 0.6% [0.4-4.4%] of CD45+ lymphocytes in normal skin (n=6) and 1.4% [1.0-2.3%] in lesional psoriasis skin (n=5) (Fig. 4d), which represents over 10 and 30 fold more, respectively, than the frequencies observed in blood (Fig. 2b).

ILC populations in normal skin (n=6) were variable between individuals (ILC1 median: 19% [9-32%], ILC2 median: 16.3% [4-69%], ILC3 NKp44− median: 46% [9-65%], ILC3 NKp44+ median: 13% [5-24%]). Figure 4e shows distribution of the different subsets, as mean frequency, in normal, non-lesional and lesional psoriasis skin. Interestingly, the overall frequency of ILC in non-lesional skin of psoriasis patients was significantly increased compared to healthy individuals (median 2.9% [2.2-5.1%], PN vs NN $p<0.05$) (Fig. 4d). Moreover, the distribution of ILC populations was highly skewed, with a significant enrichment of the NKp44+ ILC3 to a median frequency of 48% [34-54%] (PN vs NN $p<0.05$) (Fig 4f). This population also appeared to be enriched in psoriasis lesions in comparison to normal skin, although this did not reach statistical significance (median 31% [13-47%]) (Fig. 4f). However, due to the overall enrichment of ILC in lesional (PP) compared to non-lesional (PN) skin (PP median: 2,100 [500-3,600] ILC/cm^2 skin. PN median: 800 [100-1400] ILC/cm^2 skin) there was an increased overall density of NKp44+ ILC3 in each lesional (PP) sample when compared with the matched non-lesional sample (PN) (n=5, PP median 440 [250-1200] cells/cm^2 skin, PP median 260 [60-770] cells/cm^2 skin, PN vs PP $p<0.05$).

The enrichment of ILC in the skin suggests that these cells are important players in tissue immunity. The increase in the frequency of ILC and particularly the NKp44+ ILC3 subset in non-inflamed skin of psoriasis patients is particularly interesting since it indicates that these cells are not bystanders recruited to the skin following T cell activation. NKp44+ ILC3 have previously been shown to produce IL-17 and IL-22 (Bernink *et al.*), which is consistent with the trend observed of increased CD3− cytokine producing cells in PN samples (Fig. 1c and S2). The enrichment in PN samples suggests that these cells may be strategically placed and poised to start the psoriatic disease process if appropriate stimuli occur. As such, they could represent the innate counterparts to the tissue resident memory T cells of adaptive immunity which are known to reside in the skin to act as the first-line of defence in the tissue (Boyman *et al.*, 2007; Clark, 2010) and provide long term peripheral immunity (Gebhardt *et al.*, 2009; Jiang *et al.*, 2012). Activation of resident memory T cells is necessary and sufficient for the development of psoriatic lesions from human non lesional skin transplanted onto an AGR129 immunodeficient mouse (Boyman *et al.*, 2004), and skin resident ILC could contribute to provide the necessary activation signals. In this sense, ILC would have a role in initiating the psoriasis plaque, which is then sustained by proliferating T cells, which are

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present at high frequency in lesional skin. Further studies, for instance using the xenotransplant model (Boyman *et al.*, 2004), are required to elucidate ILC function in initiating the psoriasis plaque, Our findings are complementary but distinct to our previous description of the role of Vγ9Vδ2 γδ T cells in psoriasis, which are recruited to psoriasis skin under inflammatory conditions (Laggner *et al.*, 2011). Our observation that Vγ9Vδ2 γδ T cells represent only a minority of IL-17 production in non-lesional skin is now explained by the discovery of NKp44+ ILC3 as potentially major innate contributors of IL-17 and IL-22 production in conditions of skin homeostasis and inflammatory pathology.

Taken together we establish a potentially important role for NKp44+ ILC3 in the pathogenesis of psoriasis paving the way for the investigation of such cells as therapeutic targets or biomarkers in the management of psoriasis.

Materials and methods

Subjects

36 Healthy individuals (18 men and 18 women; mean age 44 years) were included in the study. 34 Psoriatic (29 men and 5 women; mean age 42 years) and 10 AD (5 men and 5 women; mean age 37 years) patients were recruited at St. John's Institute of Dermatology (Guy's and St. Thomas' Hospital), West Middlesex University Hospital and Queen Elizabeth Hospital, after examination by expert clinicians. Unless otherwise stated in Supplementary Table 2, psoriasis and AD patients were off biologic and systemic immunosuppressive therapy for at least 2 weeks. Full demographics of participants are in Supplemental Tables 1-3. Our study was conducted in accordance with the Helsinki Declaration, written informed consent was obtained from each participant, and approved by the institutional review board of Guy's and St. Thomas' Hospital (Guy's Research Ethics Committee, Reference 06/Q0704/18) and London Bridge (London Bridge Research Ethics Committee, Reference 11/LO/1962).

Sample collection

Skin biopsies (4-6mm punch biopsy) were obtained from psoriasis and AD patients from both lesional and non-lesional skin, and healthy discarded skin was obtained from plastic surgery. One psoriatic patient (P21, Supplementary Table 2 and Fig. 3) was followed during the course of therapy with adalimumab (human monoclonal antibody anti-TNF), with blood collected before and after 1, 4 and 12 weeks of therapy.

Peripheral blood mononuclear cell isolation and storage

Peripheral blood mononuclear cells (PBMC) were isolated from blood by density centrifugation over Lymphocyte Separation Medium LSM 1077 (PAA Laboratories, Pasching, Austria) and frozen in RPMI 1640 (Life Technologies, Carlsbad, CA) containing 11.25% human serum albumin (Gemini Bio-Products, West Sacramento, CA) + 10% DMSO (Sigma, St. Louis, MO) and stored in liquid nitrogen.

Skin sample processing

Skin immune cells were obtained either by spontaneous migration out of the tissue (dermal explants) or by enzymatic isolation.

Dermal explants: epidermis and dermis were separated using dispase (5mg/ml StemCell, Grenoble, France) treatment at 4°C overnight. Dermis was then cut in small pieces and cultured in complete RPMI (RPMI containing 1% penicillin/streptomycin and 10% fetal calf serum, all from Life Technologies) for 3-5 days at 37°C /5% CO2. Nonplastic adherent cells

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that had migrated out of dermis 3-5 days after culture were harvested for flow cytometric analysis.

Enzymatic isolation: epidermis and dermis were separated using EDTA treatment (15mM, Life Technologies) for 1h at 37°C and then incubated in collagenase (0.8mg/ml, Worthington, Lakewood, NJ) at 4°C overnight. For whole skin fresh cell suspensions the skin was incubated in collagenase overnight at 37°C.

Multiparameter flow cytometry

PBMC were thawed and either stimulated for intracellular cytokine production on a custom-made stimulation lyoplate (BD Biosciences, San Jose, CA) containing Phorbol 12-myristate 13-acetate (PMA), Ionomycin, and Golgi inhibitors (Monensin and Brefeldin A), at 37°C/ 5% CO2 for 5 hours and stained on a custom-made staining lyoplate (BD Biosciences) as previously described (Villanova *et al.*, 2013) or stained with liquid antibody cocktails for surface markers.

Skin cells were either stimulated with PMA (Sigma, 50-100ng/ml), Ionomycin 1μg/ml (Calbiochem, Darmstadt, Germany), Monensin (BD Bioscience, 3μM) and/or Brefeldin A (BD, Bioscience, 5μM) at 37°C/5% CO2 for 5 hours and stained with liquid cocktails for intracellular cytokine production, or stained with liquid antibody cocktail for surface marker analysis.

Lyophilized antibody present on lyoplates and liquid antibodies used are listed in Supplementary Table 4.

Intracellular staining was performed using the BD Human Foxp3 Buffer kit (BD) according to manufacturer's instructions. Dead cells were excluded from the analysis by staining with Live Dead Aqua/Yellow (Life Technologies).

Samples were acquired on a SORP Fortessa (BD Bioscience), and data analysed using DIVA software (BD) or FlowJo (Treestar, Ashland, OR).

Statistical analysis

Indicated populations were assessed for normal Gaussian distribution with D'Agostino & Pearson omnibus normality test and then analyzed by one-way ANOVA, followed by Bonferroni post test or Kruskal-Wallis, followed by Dunn's Multiple Comparison test, as appropriate, using Prism version 5.0 (GraphPad Software, La Jolla, CA). Paired lesional and non-lesional skin samples were compared using a Wilcoxon signed rank test. Unless otherwise stated mean values are given ± SEM and median values with [range]. Values of $P < 0.05$ were considered significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ILC	Innate Lymphoid Cells
AD	Atopic Dermatitis
NN	Normal skin
PN	Non-lesional skin of psoriasis patient
PP	Lesional skin of psoriasis patient
AN	Non-lesional skin of AD patient
AA	Lesional skin of AD patient
PASI	Psoriasis Area Severity Index
CLA	Cutaneous Lymphocyte-associated Antigen

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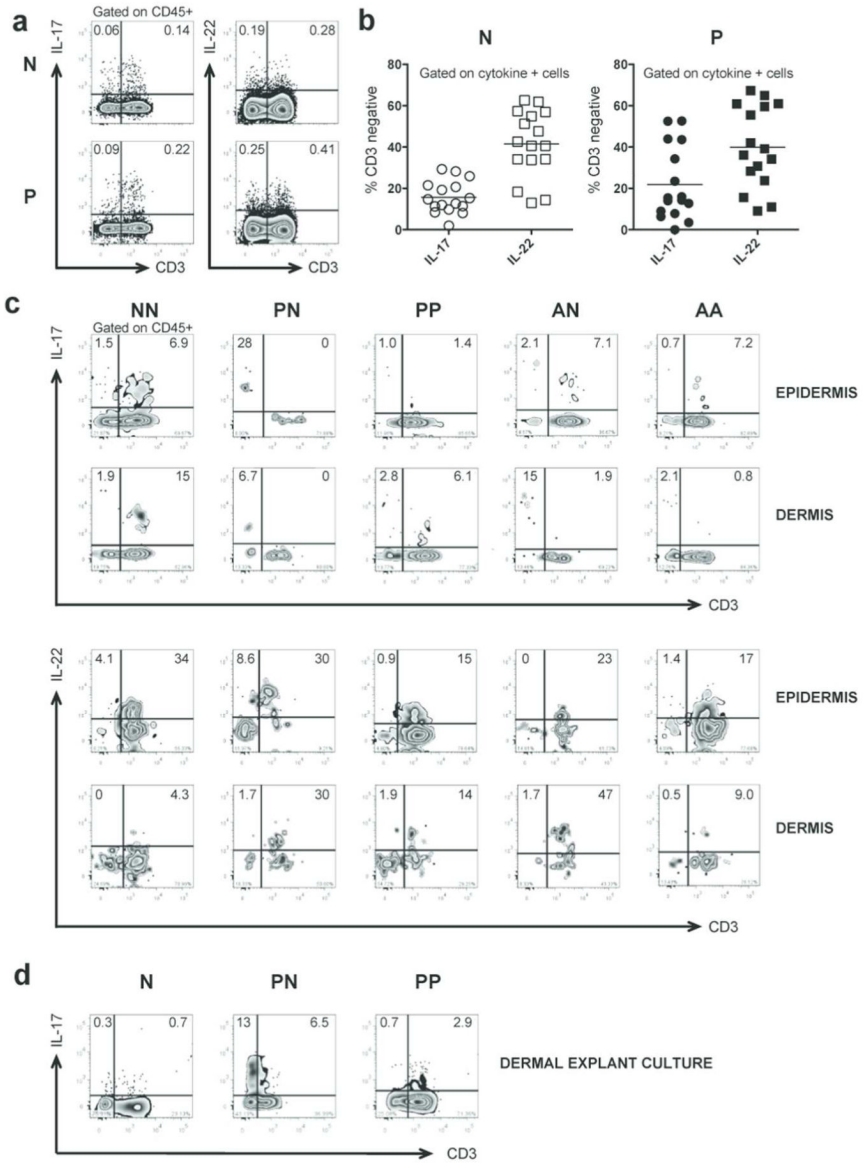

Figure 1. Identification of CD3 negative lymphocytes in blood and skin which produce IL-17 and IL-22

PBMC or skin cells from psoriasis patients (P), healthy individuals (N) or AD patients were stimulated with PMA/ionomycin and stained for surface markers and cytokines for flow cytometric analysis. **a)** Representative plots showing IL-17A and IL-22 production in PBMC from N (n=16) and P (n=16). **b)** Frequencies of CD3 negative cells within IL-17A+ or IL-22+ cells in PBMC **c)** Representative dot plots for CD3− cells producing IL-17 and IL-22 from epidermis and dermis of normal (NN, n=4 [epidermis], 5[dermis]), psoriasis non-lesional (PN, n=4 [epidermis], 3 [dermis]), psoriasis lesional (PP, n=5 [epidermis and dermis]), AD non-lesional (AN, n=5 [epidermis], 2 [dermis]), and AD lesional (AA, n=8 [epidermis], 7 [dermis) skin. **d)** Representative plots of dermal skin explants from NN (n=2), PN (n=2) and PP (n=5) skin.

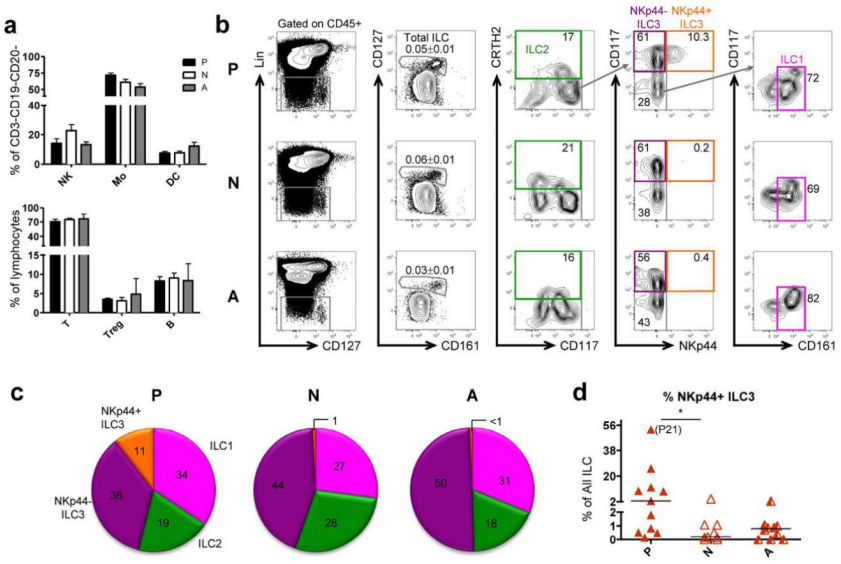

Figure 2. Deep immuno-phenotyping of PBMC shows increased frequency of NKp44+ ILC3 in blood of psoriasis patients

a) Mean frequencies ± SEM of myeloid (top) and lymphocytic (bottom) cell populations within PBMC of Psoriasis (P) (black, n=9), healthy individuals N (white, n=7) and AD patients (A) (grey, n=10). **b)** Analysis of Total ILC defined as Lin-CD127+ within live CD45+ lymphocytes in P (n=11), N (n=9) and A (n=10) (mean % ±SEM). ILC sub-populations were defined as shown in representative plots: ILC1 (pink), ILC2 (green), NKp44+ ILC3 (orange) and NKp44− ILC3 (purple). **c)** Pie charts showing average frequencies of ILC sub-populations in P, N and A. **d)** Median frequencies of NKp44+ ILC3; each filled (P), empty (N) or shaded triangle (A) represents an individual donor. Kruskal-Wallis, followed by Dunn's Multiple Comparison test was performed, *P <0.05.

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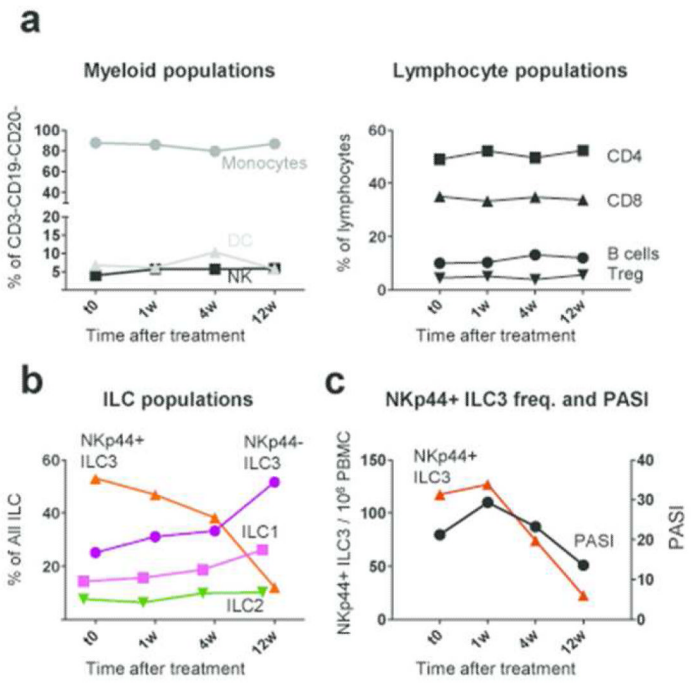

Figure 3. Reduction in NKp44+ ILC3 population during successful treatment of psoriasis with anti-TNF therapy

Case study of a psoriasis patient before and at indicated intervals after commencing therapy with the anti-TNF monoclonal antibody adalimumab. Time-course analysis showing **a)** the frequency of myeloid cell populations within CD45+/CD3−/CD19−/CD20− cells (top left); the frequency of B and T cell populations within CD45+ lymphocytes (top right); **b)** the frequency of ILC1, ILC2, NKp44− ILC3 and NKp44+ ILC3 cells within total ILCs; **c)** the absolute count of NKp44+ ILC3 within 10^6 PBMC, (left y axis) plotted against disease severity expressed as “PASI” (right y axis).

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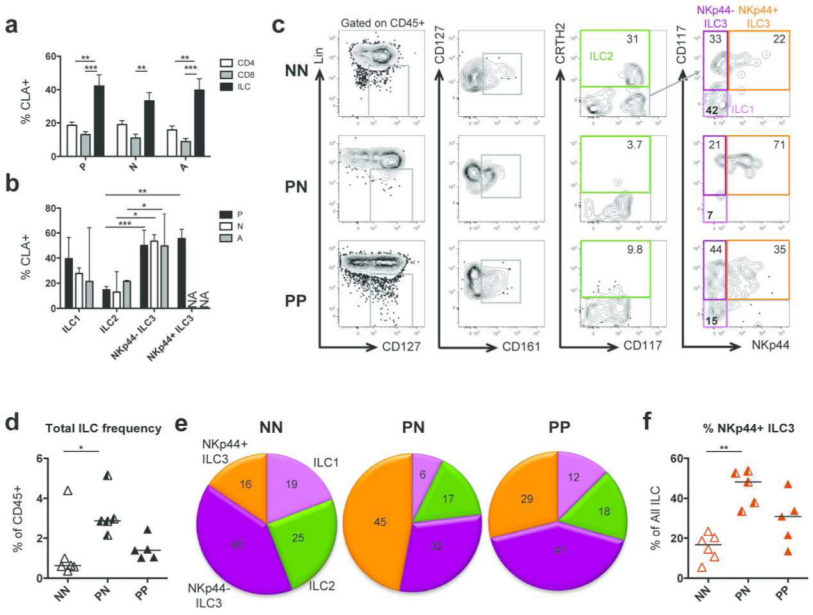

Figure 4. Circulating NKp44+ ILC3 express skin homing CLA and are enriched in skin of psoriasis patients

PBMC (a-b) and collagenase-digested skin cells (c-f) were immuno-phenotyped. **a)** Frequency (mean ± SEM) of CLA+ cells within CD4s, CD8s and ILC from psoriasis (P, n=9), healthy individuals (N, n=6) and AD patients (A, n=7). **b)** Frequency (median ± IQ range) of CLA+ cells within ILC subsets. **c)** Plots representative of NKp44+ ILC3, show gating of ILC populations in normal (NN, n=6), psoriasis non-lesional (PN, n=5) and psoriasis lesional (PP, n=5) skin. **d-f)** Total ILC frequency (d) ILC subset distribution (e) and frequency of NKp44^{+} ILC3 (f) in NN, PN and PP skin. Each open (NN), shaded (PN) and filled (PP) triangle represents an individual donor (d&f). One-way ANOVA, with Bonferroni post test (a) or Kruskal-Wallis, with Dunn's Multiple Comparison test (b,d&f), *P<0.05, **P<0.01, ***P<0.001.

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