



Published in final edited form as:

J Invest Dermatol. 2015 January ; 135(1): 311–314. doi:10.1038/jid.2014.317.

IL-17A production in human psoriatic blood and lesions by CD146+ T Cells

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Keywords

CD146; Psoriasis; Th17; Tc17; Inflammation

To the Editor:

CD146, also called melanoma cell adhesion molecule (MCAM), is a cell surface adhesion molecule on endothelial cells involved in homotypic and heterotypic cell interactions (Bardin *et al*, 2001). CD146 binding in endothelial cells (ECs) leads to a change in cellular permeability, actin distribution and redistribution of NF-kappa B p50 to the nucleus. CD146 has been shown to be present on 1–3% of circulating peripheral blood T cells in healthy humans (Elshal *et al*, 2005). CD146+ T cells have an effector memory phenotype, demonstrate up-regulation of a cluster of genes involved with adhesion, migration, homing, and inflammation, and have enhanced binding to endothelial monolayers *in vitro* (Elshal *et al*, 2007). These features of the CD146+ T cells in the peripheral circulation have led to speculation that these represent a small pool of cells primed for extravasation and/or homing of activated T cells (Elshal *et al*, 2007, Guezguez *et al*, 2007) in response to inflammatory stimuli. Circulating CD146+ T cells are elevated in several inflammatory autoimmune diseases such as sarcoidosis, inflammatory bowel disease, multiple sclerosis, connective tissue disease, and Behcet's disease and produce IL-17 (Dagur *et al*, 2011, Dagur *et al*, 2010, LaRochelle *et al*, 2012). Whether these cells play a role at the site of active inflammation in

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Conflicts of interest: none

these diseases remains unknown. Psoriasis, which is associated with increased vascular inflammation (Mehta *et al*, 2009) and access to both peripheral blood and the disease target tissue (e.g. skin), is ideal to study CD146+ T cell phenotype and function in an inflammatory condition. Here we present findings from a well-characterized patient population with psoriasis using peripheral blood samples and skin biopsies from psoriatic lesions and uninvolved skin.

Forty-seven patients with psoriasis and sixty-seven healthy controls were included in this study. Diagnosis of psoriasis was confirmed by a dermatologist and severity was measured by percentage of body surface area (BSA) involved and the validated Psoriasis Area and Severity Index (PASI). Donor demographics and characteristics are presented in Supplemental Table 1. Skin biopsies were isolated from a representative psoriatic target lesion (6 mm) and are identified as lesional psoriatic skin. Non-lesional skin biopsies were obtained from a similar body area at least 10cm away from the nearest psoriasis skin lesion. Frozen sections were obtained from skin lesions for immunofluorescence studies and all patients provided written consent in as part of an IRB-approved study (NCT01778569).

Venous blood was collected in sodium heparin vacutainers (Becton Dickinson (BD), San Jose, CA). Cells were stained and flow cytometric analysis was performed as previously described (6). Skin biopsies were digested in Collagenase IV (GIBCO BRL # 17104-019) at 5 mg/ml in RPMI 1640 for 45 min, stained, and then sorted in the same manner as peripheral blood. The following antibodies used for staining were obtained from BD: CD3, CD4, CD8, CD33, CD14, CD19, CD45, CD45-RO, CD146 (Clone P1H12). Anti-IL-17A (clone ebio64DEC17) was purchased from eBiosciences. Immunophenotyping results are expressed as means and standard errors of the mean. RNA was isolated from sorted CD146+ or CD146- T cell subpopulations using RNAquos Micro kits (Ambion) and real time PCR (QRTPCR) was performed using a 7900-sequence detector (PE-Applied Biosystems, Norwalk, CT).

Data from a single specimen were considered one experiment (n). A p-value <0.05 was considered statistically significant. Statistical analysis was performed using STATA version 12.0 (StataCorp, College Station, TX, USA).

To determine whether CD146+ T cells are prevalent in patients with a Th17 disorder, immunophenotyping was performed on fresh peripheral blood from patients with psoriasis. Psoriasis patients showed a significant elevation of circulating CD3+CD146+ T cells compared to healthy adults ($3.91 \pm 0.37\%$ vs $2.96 \pm 0.19\%$ respectively, $p=0.03$) (Figure 1A). Increased CD146 expression reached statistical significance with the circulating CD4+ T cells ($5.50 \pm 0.413\%$ in PSO vs $3.55 \pm 0.213\%$ respectively, $p < 0.0001$), (Figure 1B), but not the CD3+CD8+CD146+ T cells ($2.75 \pm 0.373\%$ in PSO vs $2.30 \pm 0.216\%$ respectively) (Figure 1C). CD146+ T cells were abundant within lesional skin biopsies, representing roughly 1/3 of the total CD4+ T and CD8+ T cell populations (Figures 1B, 1C). Immunofluorescence of frozen sections confirmed CD146+ T cells in lesional skin biopsies (Figure 1D). Lymphocytes, including CD146+ T cells, were rare in biopsies of non-lesional, unaffected skin from psoriasis patients.

To determine IL-17A production from CD146⁻ and CD146⁺ subsets of psoriatic T cells, cell suspensions from peripheral blood and lesional skin were stimulated for 3 hours with PMA and ionomycin, stained for cell surface markers, and then for intracellular IL-17A. CD146⁺ cells were the primary producers of IL-17A in lesional skin for both CD4⁺ (67.8± 9.5% p<0.005) and CD8⁺ (70.8± 11.4%, p<0.004) T cells (Figure 2A). In contrast, CD146⁺ cells accounted for ~20% of IL-17A producers in peripheral blood from both healthy adults and psoriatics. mRNA levels of IL-17A, RORc2, and CD146 were increased among unstimulated CD146⁺ T cells compared to CD146⁻ cells, in both the blood and lesional skin, with a greater elevation in the lesions (Figure 2B).

While previous studies have demonstrated increased circulating Th17 cells in psoriasis (Kagami *et al*, 2010), in this study we demonstrate that CD146⁺ T cells produce the majority of IL-17A at the active site of inflammation in psoriasis. Our study confirms previous reports of IL-17A production by CD146⁺ T cells in both healthy individuals and in patients with various autoimmune disorders and adds to those results by: 1) extending these findings to psoriasis; and 2) demonstrating that CD146⁺ T cells are important mediators of inflammation at the active site of disease. These findings suggest that CD146⁺ T cells in the circulation may represent a pool of cells with both the means to extravasate to the site of inflammation (via CD146 expression), and to mediate inflammation at a specific site. Limitations include analyzing patients with a variety of topical and systemic therapy and only studying patients with mild to moderate psoriasis. Previous studies examining this cell type in autoimmune diseases have been limited by not examining cells at the active site of inflammation – a hindrance overcome in the current work.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

None

Disclosures and Funding sources: This work was funded by Intramural Research Program at the National Heart, Lung, and Blood Institute, NIH.

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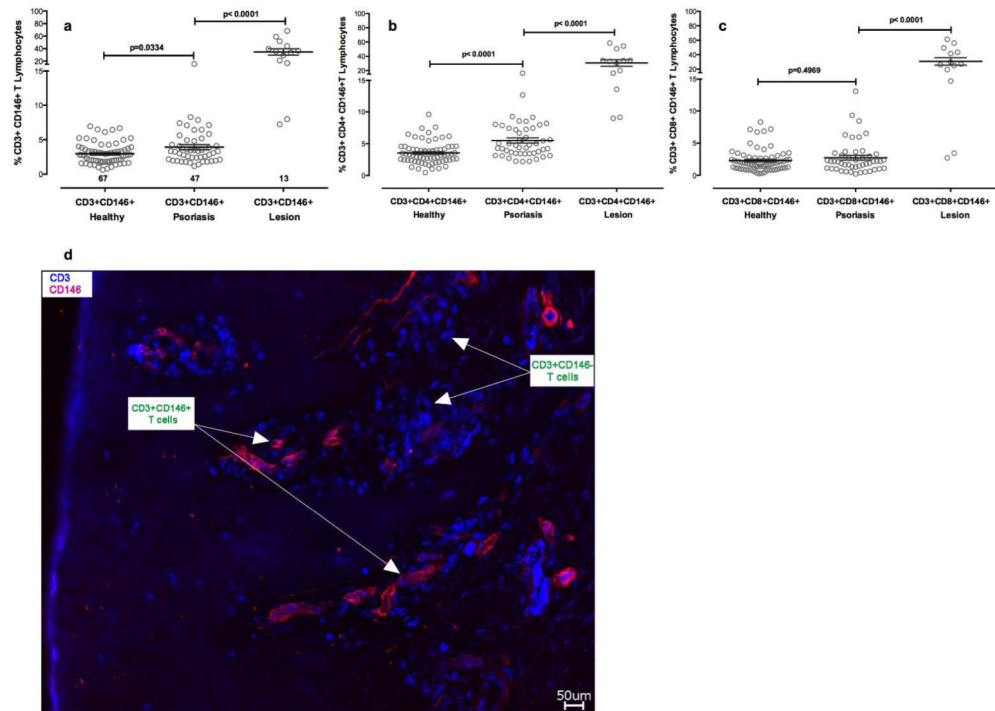


Figure 1. CD146+ T cells are significantly elevated in patients with psoriasis in the circulation and at the site of inflammation. Comparative frequencies (Mean \pm SEM) of:

Figure 1A. CD3+CD146+ T cells

Figure 1B. CD3+CD4+CD146+ T cells

Figure 1C. CD3+CD8+CD146+ T cells

Figure 1D. Immunofluorescence staining of a frozen section from a lesion biopsy stained with CD146 and CD3. The lesion stained with both antibodies demonstrated the marked infiltration of T cells expressing CD146, confirming the flow cytometry results.

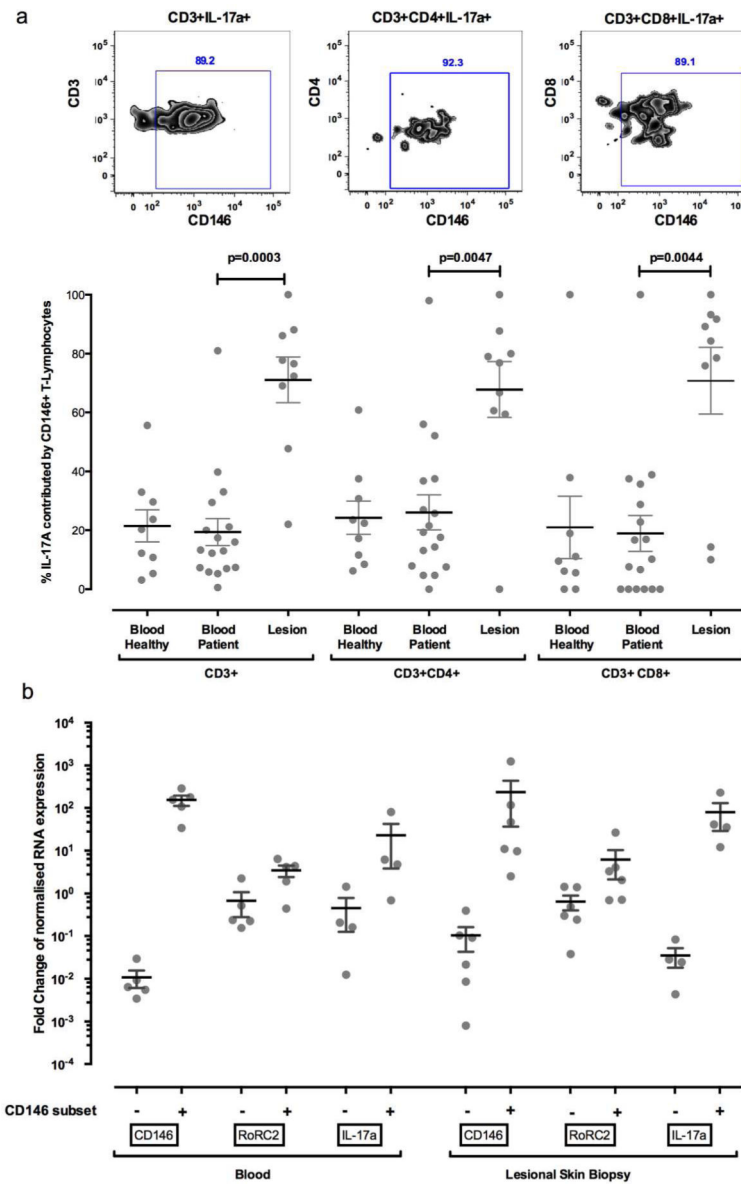


Figure 2.

Figure 2A. Percentages of IL-17A -producing T cells which are CD146 positive. The T cells and T cell subsets secreting IL-17A were gated first and then the proportion of these cells expressing CD146 were determined.

Figure 2B. Gene expression data illustrating IL-17A, RoRC2, and CD146 mRNA in CD146+ and CD146- T cells from psoriatic lesions and peripheral blood.