Human mast cells are major IL-22 producers in patients with psoriasis and atopic dermatitis

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Background: Psoriasis is a systemic inflammatory disease in which IL-17 and IL-22 levels are markedly increased in the skin and blood. The prevalent concept, using skin cells that are isolated from psoriatic plaques and examined after cell expansion and *in vitro* stimulation, is that IL-17 and IL-22 production essentially results from T cells and the rare type 3 innate lymphoid cells.

Objective: We sought to examine the cellular source of IL-17A and IL-22 at the protein and transcriptional single-cell level immediately after *ex vivo* skin cell isolation from psoriatic plaques.

Methods: Skin biopsy specimens were collected from patients with psoriasis, as well as from patients with atopic dermatitis. Cell suspensions were prepared by combining mild enzymatic digestion and mechanical dissociation and analyzed for cytokine expression without prior *in vitro* culture and stimulation. Expression of IL-17 and IL-22 was quantified at the protein and mRNA single-cell level by using flow cytometry.

Results: IL-22 is predominantly expressed by $CD3^{-}$c-Kit^{+} cells relative to $CD3^{+}$ T cells in lesional skin of patients with psoriasis and patients with atopic dermatitis. Strikingly, we identified c-$Kit^{+}Fc\varepsilon RI^{+}$ mast cells as major IL-22 producers. The proportion of mast cells that produce IL-22 ranges from 20% to 80% in patients with psoriasis or those with atopic dermatitis. Skin mast cells express IL-22 and IL-17 mRNA. Conversely, IL-17–producing T cells outnumber IL-17–producing mast cells, which also express IL-17 receptor.

Conclusion: Human skin mast cells are previously unrecognized IL-22 producers. We further established that skin mast cells express IL-17. Thus mast cells might play an important role in the physiopathology of chronic inflammatory skin disorders. (J Allergy Clin Immunol 2015;■■■:■■■-■■■.)

Key words: *Mast cells, T cells, IL-22, IL-17, psoriasis, atopic dermatitis*

Psoriasis vulgaris is a common chronic inflammatory skin disorder with a prevalence of approximately 2%. This complex disease results from dysregulation in crosstalk between environmental factors, epithelial cells, and immune cells in genetically predisposed subjects.[1,2] Over the years, the pathogenesis of psoriasis has evolved from a keratinocyte-centered to an immune-mediated disorder. Initially attributed to T_H1 cells that produce TNF-α and IFN-γ, the key role of T_H17 and T_C17 cells expressing the proinflammatory IL-17A, IL-17F is nowadays highlighted in psoriasis pathogenesis. Dendritic cell–derived IL-23 promotes the survival and expansion of pathogenic T_H17 cells.[3] Genome-wide association and genetic studies linked the TNF-α signaling pathway and the IL-23/T17–related genes to psoriasis.[4-6] The importance of these proinflammatory cytokines to psoriasis pathogenesis is now established after the clinical therapeutic success observed in patients with psoriasis treated with anti–TNF-α, anti–IL-12p40 (IL-12/IL-23 subunits), and anti–IL-17 mAbs.[7-10]

T_H17 cells can also produce IL-22, an IL-10 cytokine family member that plays a key function in skin biology. However, it is generally accepted that IL-22 can be secreted by T_H22 independent of IL-17, as well as by $CD8^{+}$ T cells (T_C22 cells). IL-22 mediates its effects in tissues by binding to its heterodimeric receptor, comprising the ubiquitously expressed receptor IL-10 receptor B and its unique receptor IL-22RA, which are exclusively expressed on epithelial cells, including keratinocytes in the skin, hepatocytes in the liver, and epithelial cells in the gut. In addition to IL-22, IL-22Ra is also able to bind IL-20 and IL-24 to activate signal transducer and activator of transcription (STAT) 1 and STAT3.[11] IL-22 in combination with IL-17 inhibits the differentiation of keratinocytes and increases their proliferation and mobility, which results in retention of nuclei in the stratum corneum (parakeratosis), epidermal hyperplasia (acanthosis), and elongation of the epidermal rete ridges (papillomatosis), the hallmarks of skin psoriatic plaques. Notably, IL-22 is abundantly expressed in lesional compared with nonlesional skin of patients with psoriasis.[12] Finally, IL-22 promotes the expression of antimicrobial peptides and therefore plays a significant role in host defense and antimicrobial protection.[13]

The involvement of innate immune cells, such as neutrophils, γδ T cells, and innate lymphoid cells, is increasingly appreciated in human psoriasis and psoriatic-like skin inflammation in mice because these cells are thought to represent an additional source of IL-17, IL-22, or both.[14-18] Moreover, mast cells have been recently

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Abbreviations used
APC: Allophycocyanin
CD: Crohn disease
FITC: Fluorescein isothiocyanate
ILC3: Type 3 innate lymphoid cell
IL-17R: IL-17 receptor
PE: Phycoerythrin
PMA: Phorbol 12-myristate 13-acetate
STAT: Signal transducer and activator of transcription

identified as IL-17 producers in psoriatic skin, although these observations are still debated.[19] In fact, mast cells are strategically positioned in the dermis and hypodermis at the interface between the external environment and the epithelium.[20-22] In response to various epithelial insults, mast cells are active participants in wound healing.[23] Moreover, numbers of IL-8–, TNF-α–, and IFN-γ–producing mast cells are increased in psoriatic skin.[24,25]

In the majority of reports studying immune cells from psoriatic skin, the function of T cells was examined by using histologic analysis of skin biopsy specimens and/or *in vitro* skin explants cultured for several days, whereby cytokine expression was measured after T-cell stimulation to enhance cytokine production. Here we present a novel method that enables the characterization of inflammatory cells from biopsy specimens of psoriatic skin in less than 6 hours, with minimal enzymatic digestion, no expansion, and no stimulation. Using this experimental approach, for the first time, we demonstrate that mast cells express IL-22 and IL-17 at the transcriptional and protein single-cell level in psoriatic plaque and lesional skin of patients with atopic dermatitis. We further show that mast cells are major IL-22 producers, whereas T cells appear to be the main source of IL-17A.

METHODS

Skin samples

Informed consent was obtained from all patients included in the study (patients with psoriasis, n = 30; patients with atopic dermatitis, n = 6). The study received the approval of an institutional ethics committee. Biopsy specimens (n = 1-3 per patient) were collected from lesional or nonlesional sites in patients with psoriasis and those with atopic dermatitis.

Skin cell isolation

Punch biopsy specimens (4 mm in diameter) were treated within less than 90 minutes of collection. Briefly, the biopsy specimen was cut into 3 pieces and enzymatically digested with 0.2 mg/mL Liberase (Roche, Mannheim, Germany) for 60 minutes at 37°C in Dulbecco modified Eagle medium (Life Technologies, Burlington, Ontario, Canada), followed by mechanical dissociation with a gentleMACS Dissociator (Miltenyi Biotech, Bergisch Gladbach, Germany). This procedure was repeated twice. Viable cells were counted by using trypan blue dye exclusion, and single-cell suspensions were immediately stained with the LIVE/DEAD Fixable Aqua Dead Cell Stain Kit at 405 nm of excitation (Molecular Probes, Life Technologies, Burlington, Ontario, Canada). Skin cell suspensions were incubated for 15 minutes at 4°C with the human IgG or anti-human CD32 blocker from STEMCELL Technologies (Vancouver, British Columbia, Canada) and stained for surface antigens for 30 minutes at 4°C. Cells were then fixed, permeabilized, and stained for intracellular cytokine expression for 30 minutes at 4°C by using the following mAbs (see Table E1 in this article's Online Repository at www.jacionline.org): Alexa Fluor 700 anti-CD3 (UCHT1), phycoerythrin (PE)–Cy7 anti-CD3 (UCHT1), PerCP anti-CD3 (UCHT1), Alexa Fluor 488 anti-CD45RA (HI100), PerCP/Cy5.5 anti-CD45RO (UHL1), allophycocyanin (APC) anti–c-Kit (104D2), Brilliant Violet 605 anti–c-Kit (104D2), PerCP/Cy5.5 anti-CD123 (6H6), PE-Cy7 anti-CD127 (A019D5), PE-Cy7 anti-CD172αβ (SE5A5), Pacific blue anti-FcεRIα (AER-37), PerCP anti-FcεRIα (AER-37), Alexa Fluor 647 anti–IL-17A (BL168), Alexa Fluor 647 anti–IL-17AR (BG-/hIL-17AR), Pacific blue anti–Lineage Cocktail (CD3, CD14, CD16, CD19, CD20, CD56, UCHT1, HCD14, 3G8, HIB19, 2H7, and HCD56), Alexa Fluor 647 IgG1κ isotype-matched control (MOPC-21), PE-Cy7 IgG1κ isotype-matched control (MOPC-21) and fluorescein isothiocyanate (FITC) IgG2bκ isotype-matched control (MPC-11) were purchased from BioLegend (San Diego, Calif). Alexa Fluor 700 anti-CD45 (2D1), APC anti–IL-17 (41802), PerCP anti–IL-17 (41802), PE anti–IL-22 (142928), PerCP IgG_1 isotype-matched control (11711), PE IgG_1 isotype-matched control (11711) and PerCP IgGb2 isotype-matched control (133303) were purchased from R&D Systems (Minneapolis, Minn). FITC anti-FcεRIα (AER-37) was purchased from eBioscience (San Diego, Calif). APC-H7 anti-CD45 (2D1) was purchased from Becton Dickinson (San Jose, Calif). FITC anti-CD3 (UCHT1) was purchased from ID Labs Biotechnology (London, Ontario, Canada).

For morphologic studies, c-$Kit^+$$Fc\varepsilon RI^+$ mast cells and $CD3^+$ T cells were purified after gating on $CD45^+$ hematopoietic viable cells with a FACSAria cell sorter. Cells were then cytocentrifuged and processed for Wright stain according to the routine procedures of the Department of Hematology at Centre Hospitalier de l'Université de Montréal.

For the FlowRNA assay, detection of IL-17, IL-22, and β-microglobulin human mRNA transcripts was performed with the QuantiGene FlowRNA assay (Affymetrix, Santa Clara, Calif), according to the manufacturer's instructions. Probes against IL-17 (type 1), IL-22 (type 1), and β_2-microglobulin (type 1 and 4) human mRNA transcripts were purchased from Affymetrix. Cytokine production was analyzed with the BD Cytofix/Cytoperm Fixation/Permeabilization Solution Kit (Becton Dickinson).

Fluorescence-activated cell sorting analysis was performed on $CD45^+$ gated hematopoietic viable cells after doublet exclusion by using forward scatter and side scatter gating strategies. Cells were analyzed with the FACSAria II and LSR II (Becton Dickinson) with FCS express flow cytometry analysis software.

Human peripheral blood memory T-cell preparation

Central memory $CD4^+$ adult T cells were purified by using the FACSAria cell sorter, and 25×10^4 cells were activated on 24-well plate-bound anti-CD3 (10 μg/mL; OKT-3; Janssen-Ortho, Toronto, Ontario, Canada) in Iscove modified Dulbecco medium (Life Technologies, Burlington, Ontario, Canada) supplemented with 10% FCS (Wisent, Saint-Bruno, Quebec, Canada) in the presence of IL-23 (10 ng/mL) and IL-1β (10 ng/mL; PeproTech, Rocky Hill, NJ) for 7 to 9 days. Primed memory T cells were restimulated for 4 hours with phorbol 12-myristate 13-acetate (PMA; 5 ng/mL; Sigma-Aldrich, St Louis, Mo) plus ionomycin (0.5 μg/mL; Calbiochem-Behring, San Diego, Calif) in the presence of monensin (3 μmol/L, Calbiochem-Behring). Unstimulated or PMA/ionomycin-stimulated T cells were stained with FITC anti-CD8 and PerCP anti-CD45RA. Intracytoplasmic cytokine protein and mRNA detection was performed by using flow cytometry. In some conditions, before human mRNA transcript detection, restimulated central memory T cells were mixed with fresh murine isolated lymph node cells at a 1:1 cell ratio and used as a negative control mRNA detection.

Statistical analysis

Paired Student *t* tests or Wilcoxon paired tests were performed, where applicable. For nonpaired data, Mann-Whitney tests or unpaired Student *t* tests with the Welch correction were used.

RESULTS

Characterization of immune cells in skin cell suspensions freshly isolated from skin biopsy specimens of patients with psoriasis

The present study aims to examine T cells and innate immune cells in plaques of patients with psoriasis immediately after skin