Six-transmembrane epithelial antigens of the prostate comprise a novel inflammatory nexus in patients with pustular skin disorders

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Background: Pustular skin disorders are a category of difficult-to-treat and potentially life-threatening conditions that involve the appearance of neutrophil-rich pustules. The molecular basis of most pustular skin conditions has remained unknown.
Objective: We sought to investigate the molecular basis of 3 pustular skin disorders: generalized pustular psoriasis (GPP), palmoplantar pustulosis (PPP), and acute generalized exanthematous pustulosis (AGEP).
Methods: Microarray analyses were performed to profile genome-wide gene expression of skin biopsy specimens obtained from patients with GPP, PPP, or AGEP and healthy control subjects. Functional enrichment, gene network, and k-means clustering analyses were used to identify molecular pathways dysregulated in patients with these disorders. Immunohistochemistry and immunofluorescence were used to determine protein localization. Quantitative RT-PCR and ELISA were used to determine transcript and secreted cytokine levels. Small interfering RNA was used to decrease transcript levels.
Results: Molecules and pathways related to neutrophil chemotaxis emerged as common alterations in patients with GPP, PPP, and AGEP, which is consistent with the pustular phenotypes. Expression of two 6-transmembrane epithelial antigens of the prostate (STEAP) proteins, STEAP1 and STEAP4, was increased in patients' skin and colocalized with IL-36γ around neutrophilic pustules. STEAP1/4 expression clustered with and positively correlated with that of IL-1, the IL-36 family proteins, and CXCL1/8. STEAP4 expression was activated by cytokines and suppressed by inhibition of mitogen-activated protein kinase kinase 1/2, whereas STEAP1 expression appeared less prone to such dynamic regulation. Importantly, STEAP1/4 knockdown resulted in impaired induction of a broad spectrum of proinflammatory cytokines, including IL-1, IL-36, and the neutrophil chemotaxins CXCL1 and CXCL8. STEAP1/4 knockdown also reduced the ability of keratinocytes to induce neutrophil chemotaxis.
Conclusion: Transcriptomic changes in 3 pustular skin disorders, GPP, PPP, and AGEP, converged on neutrophil chemotaxis and diapedesis and cytokines known to drive neutrophil-rich inflammatory processes, including IL-1 and members of the IL-36 family. STEAP1 and STEAP4 positively regulate the induction of proinflammatory neutrophil-activating cytokines. (J Allergy Clin Immunol 2017;139:1217-27.)

Key words: *Pustular skin disorders, neutrophils, inflammation, transcriptomic profiling, 6-transmembrane epithelial antigens of prostate, IL-1, IL-36, CXCL1, CXCL8*

Generalized pustular psoriasis (GPP), palmoplantar pustulosis (PPP), and acute generalized exanthematous pustulosis (AGEP) are pustular skin disorders with symptoms including the appearance of pustules and circumscribed collections of fluids and neutrophils within the epidermis. GPP is characterized by sudden and often repeated episodes of high-grade fever and generalized neutrophilic pustules. In contrast, PPP is characterized by chronic localized pustules on the palms and soles, and AGEP is characterized by eruptions occurring classically as adverse drug reactions. Despite different etiologies, these reactions are all characterized by massive influx of neutrophils into the epidermis, and these conditions can be difficult to treat and life-threatening.[1-3]

GPP has been considered a distinct form of psoriasis associated with *IL36RN* mutations and, consequently, overstimulation of the IL-36 pathways.[1] The same mutations were detected in patients with PPP and AGEP, leading to speculation that similar molecular

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Abbreviations used
AGEP: Acute generalized exanthematous pustulosis
DEG: Differentially expressed gene
FFPE: Formalin-fixed paraffin-embedded
GPP: Generalized pustular psoriasis
MEK: Mitogen-activated protein kinase kinase
PPP: Palmoplantar pustulosis
STEAP: 6-Transmembrane epithelial antigens of prostate
TLR: Toll-like receptor

alterations might underlie these disorders.[4,5] Interestingly, gene expression studies on patients with PPP provided evidence that expression of select neural genes was increased, raising the possibility that unique pathways are activated in patients with this disease.[6] However, the molecular abnormalities associated with GPP, PPP, and AGEP have not been systematically compared on a transcriptome-wide level, and common and/or distinct mechanisms driving these inflammatory conditions have remained largely elusive.

To this end, we have performed transcriptional profiling of skin biopsy specimens from patients with GPP/PPP/AGEP and healthy subjects. We report common molecular alterations, including *IL36RN*, *IL8*, and other genes affecting neutrophil chemoattraction, as well as unique pathways enriched in each disease. A novel shared feature of GPP/PPP/AGEP was found to be upregulation of the 6-transmembrane epithelial antigens of prostate (STEAP) family of proteins, which correlated with overexpression of a panel of proinflammatory cytokines. Both STEAP1 and STEAP4 were required for crosstalk between these cytokines under inflammatory conditions. These findings open up possibilities in targeting the STEAP pathways for the treatment of IL-36–associated pustular skin conditions and other disorders that share similar inflammatory profiles.

METHODS

Patient cohort

Archived formalin-fixed paraffin-embedded (FFPE) cases were identified by a search of the University of Michigan Department of Pathology database. Cases with reported diagnosis of GPP, PPP, and AGEP were identified, and the diagnosis was verified by means of slide review by a board-certified dermatopathologist (PWH) and chart review. Healthy control subjects and patients with chronic plaque psoriasis were identified in our clinic, and biopsy specimens were obtained for formalin fixation and paraffin embedding before processing and analyses. Healthy volunteers were recruited for blood draws for neutrophil isolation after providing written informed consent. All protocols were approved by the Institutional Review Board of the University of Michigan, Ann Arbor, and the study was carried out in accordance with the Declaration of Helsinki principles.

Microarray

RNA extraction was performed with an E.Z.N.A. FFPE RNA Isolation Kit (Omega Bio-tek, Norcross, Ga) by using the xylene-based extraction method, as specified by the manufacturer, and five 20-μm-thick FFPE biopsy sections. RNA was eluted into water and stored at −80°C until analyzed. Affymetrix Human Gene ST 2.1 microarrays (Affymetrix, Santa Clara, Calif) were processed at the University of Michigan Microarray Core Facility, according to the manufacturer's protocol. The raw microarray data (.CEL files) were processed in the publicly available software R (www.r-project.org) by using a modified version of the "affy" package and Human Entrez Gene custom CDF annotation version 19 (http://brainarray.mbni.med.umich.edu/Brainarra/Database/CustomCDF/genomic_curated_CDF.asp)[7] and the robust multichip average method.[8] Posthybridization quality control checks were performed by using the RNA degradation score, relative log expression, and normalized unscaled standard errors. Data were batch corrected by using an implementation of ComBat v3[9] within the GenePattern pipeline (http://www.GenePattern.org). To remove background, we calculated the median values of all probe sets and removed those probes with expression values of less than the lowest median value + 1 SD by using a custom Perl script. Gene list comparisons, generation of Venn diagrams, and enrichment of transcription factor motifs were performed on the adjusted expression data in Genomatix (www.genomatix.de) by using the default settings.[10] Biological processes linked to genes commonly altered in patients with the 3 disorders were generated by using GeneGO with DEGs common to GPP, PPP, and AGEP as input and default settings.

K-means clustering was performed with GENE-E (www.broadinstitute.org/cancer/software/GENE-E/). For K-means clustering, gene clustering was performed with default cluster numbers of 2 to 4. For each number of clustering, STEAP1 and STEAP4 coclustered with CXCL1, CXCL8, IL-1, and IL-36 family proteins (Fig 1 and see Figs E14 and E15 in this article's Online Repository at www.jacionline.org). This was further confirmed with a cluster number of 5 (see Fig E16 in this article's Online Repository at www.jacionline.org). Patient clustering was performed with default cluster numbers of 2 to 3. For each number of clustering, the group of patient-only samples (ie, without healthy subjects) correlated with high expression of the *STEAP1/4-CXCL1/8-IL1-IL36* group of genes (Fig 1 and see Fig E17 in this article's Online Repository at www.jacionline.org). This was further confirmed with a cluster number of 4 (see Fig E18 in this article's Online Repository at www.jacionline.org). Therefore a representative image for a gene cluster number of 4 and patient cluster number of 3 is shown in Fig 1. The clustering was also reiterated 20 times, and each time the above the conclusion was validated, suggesting the robustness of the method (data not shown). Functional enrichment analyses and pathway generation were performed with Genomatix, Ingenuity IPA (www.qiagen.com/ingenuity), and ClueGO.[10,11] For correlation analyses, expression values (normalized relative expression from microarray analyses) were fitted by using linear regression, and slope was tested for significant deviation from 0 (shown as *P* values). The correlation coefficients for the pairwise comparisons are shown by using *r* analysis (Pearson correlation coefficients).

Statistics

Statistical analyses of the microarrays were performed by using the significance analysis of microarrays method implemented in the MultiExperiment Viewer application.[12] Mann-Whitney and Student *t* tests were used in gene expression comparisons, as specified in the figure legends. For Fig 2, *STEAP* mRNA levels were normalized to those of *RPLP0* in each sample. Each data point represents the sample from 1 subject. Lines show the mean of the different samples in the specified group. Plaque psoriasis and PPP samples were compared with NN samples. GPP samples were compared with normal palm and sole skin specimens from 10 healthy subjects. In Fig 3 and 4, each bar shows the mean from 3 experiments. Error bars are the SEs of the mean from 3 experiments. Each treated condition was compared with the nontreated condition. In Fig 5, for the nonstimulated and IL-1β– stimulated experiments, each bar shows the mean from 3 experiments. Error bars are the SE of the mean from 3 experiments. For the IL-36α–stimulated experiments, each bar shows the mean from 4 experiments. Error bars are the SE of the mean from 4 experiments.

Keratinocyte culture, cytokine stimulations, inhibitors, RNA interference, and gene expression analyses

Normal human keratinocytes were established from healthy adults, as previously described,[13] and grown in medium 154 CF (Thermo Fisher M154CF500; Thermo Fisher, Waltham, Mass) with human keratinocyte growth supplement (Thermo Fisher S0015). Inhibitors used included the mitogen-activated protein kinase kinase (MEK) 1/2 inhibitor PD98059 (Tocris 1213; Tocris Bioscience, Ellisville, Mo), the MEK5 inhibitor Bix02189