The Spectrum of Mild to Severe Psoriasis Vulgaris Is Defined by a Common Activation of IL-17 Pathway Genes, but with Key Differences in Immune Regulatory Genes

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Mild versus severe psoriasis is often distinguished by clinical measures such as the extent of skin involvement or Psoriasis Area and Severity Index score, both of which use arbitrary boundaries. It is widely assumed that severe psoriasis involves higher levels of skin inflammation, but comparative molecular profiles of mild versus severe disease have not been performed. In this study, we used immunohistochemistry, reverse transcription PCR, and gene arrays to determine the phenotype of North American patients with mild psoriasis (n = 34, mean PASI score = 5.5) versus severe psoriasis (n = 23, mean PASI score = 23.2).

Overall, skin inflammation, defined as the sum of T-cell infiltration/activation and IL-17-mediated epidermal responses, was not higher in severe psoriasis lesions. Surprisingly, mild psoriasis was characterized by higher numbers of T cells in skin lesions, higher IL-17A expression, and stronger expression of the core psoriasis transcriptome. In contrast, severe psoriasis was characterized by stronger expression of some epidermal response genes (TGFA, CALM1, SMPD3, and IL1RL2). However, a key molecular distinction was higher expression of negative immune regulatory genes (CTLA4, CD69 and PD-L1) in mild lesions compared with severe psoriasis lesions. These data have important implications for treating psoriasis across the spectrum of disease, as well as for potential mechanisms that allow psoriasis to progress to more extensive cutaneous disease.


INTRODUCTION

Psoriasis is one of the most common T cell-mediated diseases, potentially affecting 125 million people, or nearly 3% of the world’s population (Langan et al., 2012; Langley et al., 2005; Nestle et al., 2009). Psoriasis is classified as mild versus moderate to severe, largely based on clinical evaluation tools such as the extent of skin surface affected, with greater than 10% body surface area often used to signify moderate to severe disease, and with a Psoriasis Area and Severity Index (PASI) score of 12 being a minimum for entry into many clinical trials of systemic immune modulators (Feldman, 2004; Schmitt and Wozel, 2005). Numerous conditions associated with systemic inflammation, including cardiovascular disease, obesity, and diabetes, have increased incidence in psoriasis patients, with higher risk associated with severe psoriasis (Boehncke et al., 2011; Cohen et al., 2008). Psoriatic arthritis is another inflammatory condition that appears only in psoriasis patients.

It has been widely assumed that the degree of systemic inflammation is determined by the severity of psoriasis (jiang et al., 2012; Kanelleas et al., 2011). As supporting evidence, there have been reports of positive correlation between disease severity and skin messenger RNA expression of IL-23A, IL-17, and IL-22 in moderate to severe psoriasis (Balato et al., 2014). Serum levels of T helper (Th) 1 cytokines (IL-17A, IL-22, IL-20, and IL-8), Th1 cytokines (IFN-γ, IL-12, and IL-18), and other inflammatory cytokines (tumor necrosis factor-α, vascular endothelial growth factor, fibrinogen, and C-reactive protein) have been positively correlated with disease severity (Choe et al., 2012; Jacob et al., 2003; Kanelleas et al., 2011; Lo et al., 2010; Michalak-Stoma et al., 2013; Nakajima et al., 2011; Takahashi et al., 2010). Genetic variant study indicated that activation of IL-23 and NF-κB pathways might influence disease progression to severe...
psoriasis, but no protective gene was identified in the mild disease (Nikamo et al., 2015). Thus, a commonly held view is that skin inflammation is less in mild disease, and this perception has influenced therapeutic options for mild disease that are largely centered on topical agents. However, a direct assessment of cutaneous disease severity can be derived by quantification of cellular immune infiltrates, cellular activation defined by production of disease-associated cytokines and response pathways, and the overall molecular profile of tissue disruption as defined by global messenger RNA expression patterns, as well as the resulting tissue response. To our knowledge, there are no studies at this level that directly compare cutaneous lesions of mild psoriasis vulgaris with moderate to severe disease.

In our recent study, we performed detailed cellular and molecular profiling of a mild psoriasis variant, termed small plaque psoriasis, that occurs in the Asian population versus moderate to severe psoriasis vulgaris in the Western population (Kim et al., 2016). That study showed the surprising finding that IL-17–mediated inflammation was higher in small plaque skin lesions compared with more extensive disease, and small plaque disease was also associated with higher expression of negative immune regulatory genes that might ultimately control plaque expansion. However, because of differences in ethnic backgrounds and associated genetics, it was questioned whether the findings of this study could be generalized to mild versus severe psoriasis vulgaris occurring in the Western population. Accordingly, we performed deep cellular and molecular phenotyping of mild versus moderate to severe psoriasis vulgaris in a population living in North America. This study shows “mild” skin lesions have a higher density of immune infiltrates, higher expression of IL-17 and downstream induced products, and overall higher global genomic score for molecular disease alterations compared with more “severe” disease. Skin lesions from patients with mild disease also have increased expression of negative immune regulators compared with those who have moderate to severe disease.

RESULTS
Comparing mild versus severe psoriasis skin for immune cell infiltration
According to the severity definition used in many clinical trials (Feldman, 2004; Schmitt and Wozel, 2005), a PASI score of 12 or greater is used to define (moderate to ) “severe” psoriasis. When this definition was applied to 57 North American patients with psoriasis vulgaris, 34 patients had mild psoriasis (mean PASI score = 5.5), and 23 patients had severe psoriasis (mean PASI score = 23.2). Between mild and severe psoriasis patients, there was no difference in sex, age, and duration of disease (see Supplementary Table S1 online). Consistent with previous studies (Gladman et al., 2005), severe psoriasis patients reported high prevalence of psoriatic arthritis (38.1%).

Histologic features of mild psoriasis skin were identical to those of severe psoriasis skin, including hyperkeratosis, parakeratosis, loss of granular layer, and elongated rete ridges (Figures 1a and b) (Kim and Krueger, 2015). Keratinocyte hyperproliferation (Figure 1d–f), CD11c+ myeloid dendritic cell accumulation (Figure 1i–l), and forkhead box P3 (FoxP3+) T-cell accumulation (Figure 1m–o) were not different between mild and severe psoriasis lesions (P > 0.05).

Mild psoriasis skin had an average epidermal thickness (284.9 µm); however, that was marginally lower than in severe disease (346.3 µm) (P < 0.05, Figure 1c). In contrast, CD3+ T cells were more abundant in both epidermis and dermis of mild psoriasis compared with severe disease (P < 0.05, Figure 1g–i). In addition, cytotoxic T-lymphocyte-associated protein 4 (CTLA4+) T cells were more accumulated in the epidermis of mild psoriasis compared with the epidermis of severe psoriasis (P < 0.05, Figure 1p–r).

Comparing mild versus severe psoriasis skin for disease-associated cytokine expression
We compared the expression of disease-associated cytokines in psoriasis lesional skin by reverse transcription PCR (PCR). Among 49 genes investigated, the expressions of Th17-regulated cytokines (IL-17A, IL-17F, IL-19, IL-20, IL-8, IL-33, lipocalin 2 [LCN2], and C-C motif chemokine [CCL] 11) and Th1-regulated cytokines (IFN-γ, Mx dynamin-like GTPase 1 [Mx1], 2'-5'-oligoadenylate synthetase-like [OASL], and CCL5) were consistently higher in mild psoriasis skin compared with severe psoriasis skin (False Discovery Rate [FDR] < 0.05) (Figure 2a and b). In addition, the expression of immune regulatory molecules (CTLA4, PD-L1, and CD69) was higher in mild psoriasis skin compared with severe disease (Figure 2c).

Because mild psoriasis skin showed higher expression of driver inflammatory cytokines and negative immune regulators compared with severe psoriasis skin, we further investigated the association of those cytokine expressions and disease severity (Figure 3a). The expression of driver inflammatory cytokines (IL-17A, IL-17F, IL-8, IL-33, and Mx1) decreased as the disease severity (based on PASI score) increased (P < 0.05 in both Pearson and Spearman correlations) (see Supplementary Table S2 online), and the patterns were consistent with the decreasing number of CD3+ T cells in histology of skin in inverse correlation with PASI score (Figure 3b). Similarly, the expression of negative immune regulatory molecules (CTLA4 and CD69) decreased as the disease severity increased (P < 0.05 in both Pearson and Spearman correlations) (see Supplementary Table S2). In contrast, there was no correlation between disease severity and keratinocyte (K) hyperproliferation (K16) or expression of antimicrobial peptides (S100A12) (P > 0.05).

Comparing mild versus severe psoriasis skin for global messenger RNA expression patterns and resulting tissue response
To compare mild and severe psoriasis for the global messenger RNA expression, we obtained gene expression profiles from skin biopsy tissues of mild psoriasis lesional skin, severe psoriasis lesional skin, and normal skin from healthy subjects with the Affymetrix Human Genome U133 Plus 2.0 (Affymetrix, Santa Clara, CA) Array. In principal component analysis, mild and severe psoriasis were clustered together against normal skin by principal component 1 (Figure 4a). Unsupervised hierarchical clustering also clustered mild psoriasis and severe psoriasis together against normal skin by root node (Figure 4b). Differentially expressed genes (DEGs) of mild and severe psoriasis, defined by

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contrasting lesional psoriasis skin and normal skin, displayed similar distribution (Figure 4c), and the expressions were highly correlated (correlation coefficient \( r = 0.90, P < 0.01 \)) (Figure 4d). Among genes with greater than 2-fold change (FDR < 0.05), 57.6% of DEGs were either elevated or decreased in both mild and severe psoriasis (Figure 4e).

However, distinctions were also drawn between mild and severe psoriasis. Mild and severe psoriasis were separately clustered by principal component 2 in principal component analysis (Figure 4a). The second node of unsupervised hierarchical clustering also clustered mild and severe psoriasis separately (Figure 4b). Thus, we further investigated different expression levels of individual DEGs between mild and severe psoriasis for resulting tissue response (Table 1 and see Supplementary Table S3 online). DEGs involved in T-cell activation (PRKCA), T<sub>H</sub>17-regulated cytokines (IL17A, IL19, and CXCL1), and T<sub>H</sub>1-regulated cytokines (IL12RB2 and IRF7) were highly expressed in mild psoriasis compared with severe psoriasis (FDR < 0.05) (Table 1). In contrast, DEGs involved in epidermal hyperplasia (TGFA, CALM1, and SAMPD3) (Checa et al., 2015; Mizumoto et al., 1985), epithelial IL-36 receptor (IL1RL2) (Blumberg et al., 2007; Dietrich and Gabay, 2014; Lowes et al., 2013), and systemic autoimmunity (FCCGR3B) (Fanciulli et al., 2007) were highly expressed in severe psoriasis compared with mild disease (FDR < 0.05).

The expression pattern of DEGs involved in negative immune regulation showed similar trends to T-cell activation and T<sub>H</sub>17- and T<sub>H</sub>1-regulated cytokines. IKBKE, FAIM3, and PD1 were expressed higher in mild psoriasis compared with severe psoriasis (FDR < 0.05). IDO1, CD69, CTLA4, and PDCD11 showed trends of higher expression in mild psoriasis compared with severe disease, although the difference was not significant (FDR > 0.05). Overall, tissue response of mild psoriasis showed higher expression patterns of T-cell activation, T<sub>H</sub>17-regulated cytokines, T<sub>H</sub>1-regulated cytokines, and T<sub>H</sub>17-regulated cytokines compared with severe disease (FDR < 0.05). In contrast, DEGs involved in negative immune regulation showed similar trends to T-cell activation and T<sub>H</sub>17- and T<sub>H</sub>1-regulated cytokines.
cytokines, and negative immune regulation compared with severe disease.

Comparing mild versus severe psoriasis skin for disease-response pathway activation

To compare mild and severe psoriasis for disease-response pathway activation, gene set variation analysis (GSVA), a method that produces a score of activity for a set of genes or pathway for each sample, was performed by averaging z scores of expression values over all genes in a given pathway (Lee et al., 2008). Using GSVA, we first examined individual patients’ expression of psoriasis transcriptome, which has been established by previous studies of moderate to severe psoriasis (Bowcock et al., 2001; Gudjonsson et al., 2009; Jabbari et al., 2011; Suárez-Farina et al., 2012; Suárez-Farina et al., 2010; Tian et al., 2012; Yao et al., 2008). Both mild and severe psoriasis skin highly expressed psoriasis transcriptome, and GSVA scores for psoriasis transcriptome were significantly higher in mild psoriasis compared with severe psoriasis (Figure 5a). For example, the GSVA score for up-regulated genes in meta-analysis derived psoriasis transcriptome (MAD3 in Figure 5a) was 56.6 in mild psoriasis and 48.7 in severe psoriasis, and the difference of 7.9 was statistically significant (FDR = 0.005). Higher GSVA scores in mild disease were consistently observed across all the up- and down-regulated gene sets for psoriasis transcriptome (FDR < 0.05) (Figure 5a) (Bowcock et al., 2001; Gudjonsson et al., 2009; Jabbari et al., 2011;
Figure 3. The association of disease severity (PASI) and disease-associated cytokine expression in the skin. 
(a) Heatmap displaying Spearman correlation between PASI score and disease-associated cytokine expression in the skin. 
(b) Pearson correlation between PASI score and CD3^+ T-cell accumulation (red), pathogenic driver molecule expression (IL-17A and Mx1, red), and negative immune regulator expression (CTLA4 and CD69, blue) in the skin. As a control, no correlation between PASI score and keratinocyte hyperproliferation (K16) is observed (black). Gene expression: log_2 conversion of messenger RNA expression normalized to human acidic ribosomal protein (HARP). PASI: Psoriasis Area and Severity Index.
Suárez-Fariñas et al., 2012; Suárez-Fariñas et al., 2010; Tian et al., 2012; Yao et al., 2008).

To understand the paradox of higher psoriasis transcriptome in mild disease, we searched for immune response pathways from Molecular Signatures Database (available at http://software.broadinstitute.org/gsea/msigdb) that differentiate mild and severe psoriasis. GSVA scores for pivotal immune response pathways underlying psoriasis pathogenesis (TH17-, TH1-, and TH22-regulated pathways) were higher in mild psoriasis compared with severe psoriasis (FDR < 0.05) (Chiricozzi et al., 2011; Chiricozzi et al., 2014; Suárez-Fariñas et al., 2012). In addition, GSVA scores of regulatory T-cell (Treg) signatures (extrathymic Treg development, Treg vs. effector T-cell signature) (Layland et al., 2010; Prots et al., 2011) and FAS signaling pathway were higher in mild psoriasis compared with severe disease (FDR < 0.05) (Figure 5b). Those immune pathway findings were consistent with reverse transcription PCR experiment data (Figure 2) and global messenger RNA expression profiles (Table 1).

DISCUSSION

The results of our study suggest that psoriasis vulgaris has a highly stable tissue phenotype for its core IL-17 inflammatory axis (Figure 2 and Figure 5b) and for tissue alterations that define psoriasis histopathology (Figure 1), irrespective of disease severity. Thus, focal skin regions of pathology (psoriasis plaques) are overall very similar between mild and severe disease, and this similarity is well illustrated by GSVA scores for psoriasis transcriptome (Figure 5a) and by principal component analysis (Figure 4a) that shows variation in principal component 1 to be virtually identical between mild and severe disease. Perhaps, paradoxically, T-cell infiltration and IL-17 expression are actually higher in skin lesions of mild disease (Figure 3b).

However, distinctions between mild and severe disease can be drawn by secondary variation in expression of a subset of genes (principal component 2 axis of principal component analysis in Figure 4a and the second node of unsupervised hierarchical clustering in Figure 4b) and by quantitative differences in expression of some genes (Table 1 and see Supplementary Table S3). One striking difference is higher expression of negative immune regulatory genes in mild psoriasis (Figure 2c, Figure 3b in blue, and Table 1). A related difference is that although T-cell density is higher in plaques of mild psoriasis vulgaris (Figure 1), the overall numbers of infiltrating T cells across all plaques in moderate to severe disease will be higher and proportional to the extent of skin surface affected. Thus, one can hypothesize that the progression of psoriasis from mild disease, displaying relatively stable size and relatively few lesions, to severe disease, with large skin areas affected, may be enabled by increased T-cell proliferation and thus the creation of more skin-homing memory T cells. In turn, T-cell expansion may be enabled by having less effective immune regulation in patients that develop more extensive psoriasis lesions.

The concept of dysfunctional regulatory T cells in psoriasis stems from reduced functional activity of Tregs isolated from peripheral blood of psoriasis patients (Chen et al., 2008; Soler et al., 2013; Sugiyama et al., 2005; Viglietta et al., 2004). FoxP3+ T cells are relatively abundant in skin lesions, but functional studies have been rarely conducted on this Treg population with epidermal or dermal cell suspensions (Soler et al., 2013; Sugiyama et al., 2005). However, a recent study has found that many negative immune regulators, including checkpoint inhibitors, have very low expression in psoriasis lesions from patients with moderate to severe disease compared with those who have acute immune reactions that undergo resolution (Gulati et al., 2015). In fact, a striking difference in expression of the FAS signaling pathway (Figure 5b)
and CTLA4 expression (Figures 1 and 2) were detected in this study, with much lower expression in severe lesions. Also, the level of CTLA4 expression had a highly significant correlation with the PASI score in our patient cohorts (Figure 3b). Perhaps low-level expression of immune checkpoints, combined with constitutive expression of newly identified psoriasis auto-antigens, cathelicidin antimicrobial peptide (LL37) (Lande et al., 2014) and ADAMTS-like protein 5 (ADAMTSL5) (Arakawa et al., 2015), create the opportunity for ongoing T-cell activation by these antigens presented by dendritic antigen-presenting cells that are also highly abundant in psoriasis lesions. (CD11c+ myeloid dendritic cells are highly abundant in mild and severe lesions, as shown in Figure 1).

These results have implications for the classification of skin dysfunction across the spectrum of mild to severe disease, as well as for the best treatment options for psoriasis lesions. The current classification scheme of mild versus moderate to severe psoriasis has been drawn using pragmatic criteria that stem from the high potential toxicity of agents used previously to treat extensive psoriasis, such as cyclosporine or methotrexate (Krueger et al., 2000). It was thus viewed that topical agents had the best risk/benefit ratio for treating mild disease, and nontopical agents with a higher risk were reserved for patients with greater than 10% of body surface area affected, based on the impracticality of treating extensive lesions with topical drugs. This schema has carried forward into treatment of psoriasis with biologic immune modifiers, but advances have been made with more selective cytokine antagonists, and the toxicity profile of these agents is far less than that of earlier drugs, and the effectiveness is much higher than that of available topical agents to treat psoriasis. Because even mild disease carries increased risk of comorbid conditions such as cardiovascular disease, metabolic syndrome/diabetes, and psoriatic arthritis (Armstrong et al., 2013; Gelfand et al., 2006; Gisondi et al., 2007; Kremers et al., 2007; Neimann et al., 2006), one might argue that in some patients mild disease might be appropriately treated with low-toxicity systemic agents, particularly if benefit could be shown for systemic inflammation pathways that drive comorbid disease development.
The high expression of immune checkpoints in mild disease might also create therapeutic opportunity for re-establishing immune tolerance to psoriatic autoantigens, if agents are used that can dramatically reduce antigen-reactive T-cell clones and decrease expression of autoantigens that are regulated directly or indirectly via IL-17 and feed-forward pathways. The potential for long-term disease control without continuous immune suppression is suggested by a recent study with BI655066, an IL-23 monoclonal antibody, that showed that a single dose induced disease clearing (PASI100 response) in a subset of patients who were stable for more than 44 weeks after the treatment dose without any other concurrent treatment (Krueger et al., 2015). Because the effect on the disease is far longer than five half-lives of the antibody, we speculate that immune tolerance might have been induced, although this might be only transiently. Much more work is needed to understand this result, but other recent work has emphasized the lineage plasticity of TH17 T cells, which under some conditions may be induced to become Tregs or even TH1 T cells (Muranski and Restifo, 2013; Soler and McCormick, 2011). Thus future work needs to be directed to immune checkpoints, immune tolerance, and modulation of effector versus regulatory cell networks in psoriasis across its severity spectrum.

MATERIALS AND METHODS
Detailed statistical description and the list of real-time PCR primers are available in the Supplementary Materials and Methods online.

Study design and skin biopsy samples
This study was designed to investigate skin biopsy tissues of mild to severe psoriasis patients. Skin biopsy tissues were obtained from patients 18 years and older with active psoriasis vulgaris lesions who were living in the United States and Canada. Skin biopsy tissues were obtained from patients enrolled in two clinical trials before initiating any treatment (clinical trial numbers: NCT00844363 and NCT02078297). In both clinical trials, skin biopsy tissues were obtained in accordance with the Helsinki Declaration and approved by the institutional review boards. Written informed consent was obtained from all patients, and skin biopsies were performed at a representative psoriatic plaque of each patient. All the experiments with skin biopsy tissues were commonly conducted concurrently at the Rockefeller University laboratory for investigative dermatology. In total, 57 lesional skin biopsy tissues from psoriasis patients and 6 normal skin biopsy tissues from healthy subjects were studied.

Immunohistochemical analyses
For immunohistochemical analyses of psoriasis skin biopsy samples, half the biopsy samples were embedded in optimal cutting temperature compound (Sakura Finetek, Torrance, CA) and frozen atop a dry ice bath. For hematoxylin and eosin staining, the sections were stained with hematoxylin (Fisher Scientific, Pittsburgh, PA) and Shandon eosin (Fisher Scientific). For immunostaining, frozen sections of skin biopsy samples were dried at room temperature and then fixed for 2 minutes in acetone. Next, the samples were blocked with 10% normal serum of the species in which the secondary antibody was made, and then incubated with the appropriate primary antibody. Primary antibodies used in this study were all mouse antibodies: K16 (Bio-Rad, Hercules, CA; clone LL025, dilution 1:40), CD3 (DAKO, Carpinteria, CA; clone F7.2.38, dilution 1:50), CD11c (BD Biosciences, San Jose, CA; clone Bly6, dilution 1:100), FoxP3 (Abcam, Cambridge, MA; clone 236A/E7, dilution 1:100), and CTLA4 (LifeSpan BioSciences, Seattle, WA; clone LS-C35849, dilution 1:100). Biotin-labeled horse
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Raw microarray data of mild and severe psoriasis have been deposited in NCBI's Gene Expression Omnibus together and are accessible through accession number GSE 78097.

AUTHOR CONTRIBUTIONS
JK designed the study and established and performed all the experiments and analysis. RB collected samples and associated phenotype data. JL performed RNA isolation and isolation of total RNA from skin biopsy samples. JGK supervised and co-designed the study and wrote the paper.

REFERENCES


