Intrinsic Defect in Keratinocyte Function Leads to Inflammation in Hidradenitis Suppurativa

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Hidradenitis suppurativa (HS) is a chronic, inflammatory, debilitating, follicular disease of the skin. Despite a high prevalence in the general population, the physiopathology of HS remains poorly understood. The use of antibiotics and immunosuppressive agents for therapy suggests a deregulated immune response to microflora. Using cellular and gene expression analyses, we found an increased number of infiltrating $CD4^+$ T cells secreting IL-17 and IFN-γ in perilesional and lesional skin of patients with HS. By contrast, IL-22-secreting $CD4^+$ T cells are not enriched in HS lesions contrasting with increased number of those cells in the blood of patients with HS. We showed that keratinocytes isolated from hair follicles of patients with HS secreted significantly more IL-1β, IP-10, and chemokine (C-C motif) ligand 5 (RANTES) either constitutively or on pattern recognition receptor stimulations. In addition, they displayed a distinct pattern of antimicrobial peptide production. These findings point out a functional defect of keratinocytes in HS leading to a balance prone to inflammatory responses. This is likely to favor a permissive environment for bacterial infections and chronic inflammation characterizing clinical outcomes in patients with HS.

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INTRODUCTION

Hidradenitis suppurativa (HS), also known as acne inversa, is defined as a chronic, inflammatory, recurrent, debilitating, follicular skin disease that presents with painful, inflamed lesions in the apocrine gland-bearing areas (Jemec, 2012). Its estimated prevalence ranges from 1% to 4% (Revuz et al., 2008; Vinding et al., 2014).

The pathogenesis of HS is still poorly understood and is probably multifactorial. In a small number of cases, the disease has been linked to chromosome 1p21.1-1q25.3 and mutations of the γ-secretase complex (Gao et al., 2006; Pink et al., 2011; Wang et al., 2010). Smoking, obesity, and hormonal influences may be pathogenic factors (Canoui-Poitrine et al., 2009). HS affects predominantly occluded skin areas that are rich in terminal hair follicles (HFs) and apocrine gland. A special feature of HS appears to be the formation of keratin-filled epidermal cysts. Abnormalities in terminal HF homeostasis may result in instability of outer root sheath cells (ORS) and formation of keratin-rich epidermal cysts. Danger-associated motif patterns released by ruptured epidermal cysts exposing keratin fibers may stimulate inflammasome-mediated innate immunity (van der Zee et al., 2012).

The role of bacterial infections seems to be critical in HS and is supported by the observation that HS can be improved by antibiotic therapy (Gener et al., 2009; Join-Lambert et al., 2011; Mendonca and Griffiths, 2006). Of note, recent studies have shown different profiles of skin opportunistic bacterial infections in patients with HS (Guet-Revillet et al., 2014; Jahns et al., 2014) underlying the predisposing condition of the host to be harmful. To support this, a recent meta-analysis on HS bacteriology showed that despite that a common bacterial strain cannot be isolated from lesions, coagulase negative streptococci and mixed *anaerobic* bacteria are the most common bacteria isolated from lesions (Ring et al., 2015).

Regulation of skin responses to infection involves a complex interplay between antimicrobial peptides (AMPs) and cytokines produced by keratinocytes and local immune cells. AMPs also exert an immunomodulatory effect in the skin in cytokine production, chemoattraction of neutrophils, antigen presentation, and wound healing (Gallo and Hooper, 2012).

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Abbreviations: AMP, antimicrobial peptide; hBD-2, human-β-defensin-2; HD, healthy donors; HF, hair follicle; HS, hidradenitis suppurativa; MDP, muramyl dipeptide; ORS, outer root sheath cells; PBMC, peripheral blood mononuclear cell; qPCR, quantitative reverse transcriptase in real time-PCR; Th, T helper cells

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Some studies have suggested a defect of AMP production (Dreno et al., 2012; Hofmann et al., 2012; Wolk et al., 2011) and subsequently the production of IL-17 and IL-22, produced by T helper 17 cells (Th17) and/or Th22 cells in HS skin (Wolk et al., 2011).

Despite these studies, we still have a limited understanding of how a local defect in the control of bacterial infection and/or an inflammatory activation unfolds in patients. In particular, the primum movens of the initiation of the pathophysiology process remains poorly understood.

In this study, we observed an increased frequency of functional $CD4^+$ T cells secreting IL-17 or IL-22 in the blood of patients with HS compared with healthy donors (HD). However, we did not observe an increased frequency of infiltrating $CD4^+$ T cells secreting IL-22 in HS skin lesions, but we found an increased frequency of infiltrating $CD4^+$ T cells secreting both IL-17 and IFN-γ compared with healthy skin. Transcriptome analysis performed on whole lesional skin confirmed these results. We showed that keratinocytes isolated from HFs of patients with HS presented a distinctive pattern of AMP expression. They constitutively exhibit an inflammatory profile. After pattern recognition receptor stimulation, these cells produced higher amount of IL-1β leading to an increased production of proinflammatory cytokines. This profile severely contrasts with keratinocytes from HD. Globally, these results point out a primary dysfunction of keratinocytes from patients with HS characterized by a defect in enhancing a tissue protective response, but rather a chronic inflammatory response, to microbial products leading to a perpetual inflammation at sites of infection in skin lesions.

RESULTS

Frequencies of $CD4^+$ T cells secreting IL-17 or IL-22 are increased in the blood of patients with HS

We looked at the frequencies of IL-17, IL-22, and IFN-γ secreting T lymphocytes in peripheral blood mononuclear cells (PBMCs) from patients with HS (n = 18) and HD (n = 13) after phorbol myristate acetate/ionomycin stimulation. The frequencies of $CD4^+IL\text{-}17A^+$ and $CD4^+IL\text{-}22^+$ T cells were significantly higher in HS than HD (median [interquartile range]: 2.1 [1.3−3.5%] vs. 1.2 [0.5−1.8%] and 1.3 [0.7−1.9%] vs. 0.5 [0.3−1.1%], respectively; $P < 0.05$ for all comparisons), with no difference in frequency of $CD4^+IFN\text{-}\gamma^+$ T cells (Figure 1a) and $CD8^+IFN\text{-}\gamma^+$ T cells (data not shown).

To better characterize IL-17- and IL-22-secreting T cells and their skin homing capacity, we analyzed the expression of chemokine receptors CCR4, CCR6, and CCR10 involved in skin homing in peripheral $CD4^+$ T cells. The $CCR4^+CCR6^+CCR10^-$ pattern defines Th17 cell subsets and the $CCR4^+CCR6^+CCR10^+$ pattern Th22 cell subsets (Duhen et al., 2009; Sallusto et al., 2012). The pattern of T-cell expression of chemokine receptors did not differ between patients with HS and HD (data not shown). These results prompted us to focus on cytokines production by $CCR4^+CCR6^+CCR10^-CD4^+$ T cells and $CCR4^+CCR6^+CCR10^+CD4^+$ T cells. Patients with HS displayed a higher frequency of IL-17-secreting cells in the $CCR6^+CCR10^-$ T cell subsets and in the $CCR6^+CCR10^+$ T cell subsets ($P < 0.05$). The same trend was observed for IL-22-secreting cells, without reaching significance (Figure 1b). Next, the chemotaxis capacity of T cells from patients with HS in response to chemokines was monitored using a transwell assay. We used CCL20 as a CCR6 ligand, CCL27 as a CCR10 ligand, and CXCL12 as a positive control. The chemotactic index (proportion of migrated $CD4^+CCR4^+$ cells in the lower chamber among $CD4^+CCR4^+$ cells laid in the top well) did not differ between patients with HS and controls (data not shown).

We measured cytokine production in PBMCs after in vitro stimulation with *Staphylococcal* enterotoxin B, heat-killed *Candida albicans*, and *Staphylococcus aureus*. Production of IFN-γ, IL-17, and IL-22 in supernatant was higher in HS than HD after *Staphylococcal* enterotoxin B stimulation (all $P < 0.05$) (Figure 1c). On stimulation of PBMCs with *C. albicans* or *S. aureus*, levels of IFN-γ, IL-17, and IL-22 were consistently higher, although not always significantly, in HS as compared with HD (Figure 1c). Therefore, patients with HS displayed a high frequency of IL-17- and IL-22-secreting T cells in the blood compared with HD.

Characterization of inflammatory infiltrate in HS skin

We analyzed selected cytokine and chemokine gene expression in lesional skin biopsies from patients with HS and skin samples from HD. As assessed by quantitative reverse transcriptase in real time-PCR (QPCR) on whole lesional skin, expression of *IFN-γ*, *IL-17*, *IL-10,* and *CCL-20* was significantly increased in patients with HS compared with HD. We observed that *IL-22* was not upregulated in HS (Figure 2a) and that CCL27 expression was significantly decreased in HS lesions (0.26 vs. 0.76 arbitrary units; $P < 0.05$).

To characterize T-cell infiltrates in the skin, we analyzed T-cell populations isolated from lesional and nonlesional skins of seven patients with HS and from five HD. After mitogenic stimulation, a significantly higher frequency of IL17-producing $CD4^+$ T cells in HS lesional (median: 19.2%) as well as in perilesional skin (10.0%) was observed when compared with the skin of HD (4.8%), whereas the frequency of IL-22-producing $CD4^+$ T cells remained low (HD: 1.1%, HS lesions: 1.5% and HS perilesion: 2.5%) (Figure 2b). IFN-γ-producing $CD4^+$ T cells were significantly more frequent in lesions (24.1%) than in perilesions (6.1%) and HD skins (4.8%). Interestingly, the $CD4^+$ T-cell population that produced both IFN-γ and IL-17 was significantly increased in lesional HS (3.7%) and in perilesional skin (1.1%) compared with healthy skins (0.1%) (Figure 2b).

To further characterize inflammatory pathways in HS lesions, gene array analysis on messenger RNA isolated from the skin and blood from patients with HS and HD was performed. Unsupervised clustering analysis did not show significant differences in the blood between patients with HS and HD (data not shown). By contrast, analysis of key differentially regulated genes in the skin strengthened the observed results. Indeed, IL-17 and IFN receptor signaling pathways ranked first and second, respectively, among the most significant canonical pathways enriched with differentially expressed genes in HS lesions compared with healthy skins (Figure 3a). The IL-17 signaling pathway is known to enhance the expression of S100A7, S100A8, S100A9, human-β-defensin-2 (hBD-2), CXCL1, and IL-8 all significantly upregulated in HS.