Sebaceous Gland-Rich Skin Is Characterized by TSLP Expression and Distinct Immune Surveillance Which Is Disturbed in Rosacea

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The microbial community exhibits remarkable diversity on topographically distinct skin regions, which may be accompanied by differences in skin immune characteristics. Our aim was to compare the immune milieu of healthy sebaceous gland-rich (SGR) and sebaceous gland-poor skin areas, and to analyze its changes in an inflammatory disease of SGR skin. For this purpose, immunohistochemical, immunocytochemical, and quantitative real-time PCR analyses of thymic stromal lymphopoietin (TSLP) and other cytokines, phenotypic immune cell markers and transcription factors were carried out in samples from sebaceous gland-poor, SGR skin and from papulopustular rosacea. TSLP mRNA and protein production was also studied in cultured keratinocytes. In SGR skin, higher TSLP expression, dendritic cell appearance without prominent activation, and T cell presence with IL-17/IL-10 cytokine milieu were detected compared with sebaceous gland-poor skin. Linoleic acid, a major sebum component, was found to induce TSLP expression dose-dependently in keratinocytes. In papulopustular rosacea, significantly decreased TSLP level and influx of inflammatory dendritic cells and T cells with IL-17/interferon-γ cytokine milieu were observed. According to our results, SGR skin is characterized by a distinct, noninflammatory immune surveillance, which may explain the preferred localization of inflammatory skin diseases, and can influence future barrier repair therapeutic concepts.

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INTRODUCTION

As an outstanding discovery of recent years, the microbial community has been shown to exhibit remarkable differences on topographically distinct skin areas (Grice et al., 2009; Grice and Segre, 2011). It has been demonstrated that colonization of these bacteria is dependent on the physiology of the skin site, as specific bacteria are being associated with moist, dry, or sebaceous microenvironments, and the diversity of the chemical milieu in which these microbial communities live was also described (Bouslimani et al., 2015; Costello et al., 2009; Gao et al., 2007; Grice et al., 2009; Grice and Segre, 2011).

High-scale diversity of the microbiota was not only described on the skin barrier surface, but distinct sections of the gut are also known to be colonized by heterogeneous microbiota, which is associated with the different anatomical and physiological features of these sites (Eckburg et al., 2005). Besides the diversity of microbiota, recent studies indicated a mutual relationship between the host and these microorganisms, because they play an important role in tissue homeostasis and local immunity (Belkaid and Segre, 2014; Maranduba et al., 2015; Naik et al., 2015). These assume the possibility that the level of immune activation may differ in distinct barrier surfaces, which has been already indicated in the gut. For example, thymic stromal lymphopoietin (TSLP), one of the major epimmunomes (epithelial cell-derived molecules that can instruct immune cells), was detected only in particular gut sections, with its highest, constitutive expression in colonic epithelial cells (Rimoldi et al., 2005; Swamy et al., 2010). This protein is involved in the development of tolerance to commensal microflora through modulation of dendritic cell (DC) functions in the

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Abbreviations: AD, atopic dermatitis; DC, dendritic cell; IHC, immunohistochemistry; KC, keratinocyte; PPR, papulopustular rosacea; RT-PCR, quantitative real-time PCR; SGP, sebaceous gland poor; SGR, sebaceous gland rich; TARC, thymus- and activation-regulated chemokine; Th, T helper; TSLP, thymic stromal lymphopoietin; Treg, regulatory T cell

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gut. The tolerogenic role of TSLP is supported by recent studies where decreased TSLP level and altered microbial composition were found in Crohn's disease (Podolsky, 2002; Round and Mazmanian, 2009). Until now, TSLP in the skin was only described under inflammatory conditions, such as atopic dermatitis (AD) and psoriasis, and its only known function in this organ so far is the promotion of T helper (Th)2 polarizing DCs (He and Geha, 2010).

In this study, we asked the question whether the above topographical differences in skin microbiota and physiology can also be accompanied by topographical differences in skin immune activity and TSLP (epimmunome) production. The possibility that the skin immune system is characterized by distinct functional tuning on different skin regions was not challenged until now in the literature.

RESULTS

TSLP protein is constitutively expressed in SGR healthy skin, but almost absent from SGP healthy skin

To detect TSLP protein in topographically different skin regions, biopsies from sebaceous gland-poor (SGP; representing dry areas) and sebaceous gland-rich (SGR; representing seborrheic areas) healthy skin were obtained. Lesional skin of patients with severe AD was used as a positive control for TSLP staining. To confirm immunohistochemistry (IHC) results three different antibodies against TSLP were used (Figure 1a). In AD samples, strong TSLP positivity was detected in the granular and corneal but not in the basal and suprabasal layers of the epidermis. In all SGR skin biopsies, high TSLP expression was detected with all three anti-TSLP antibodies in the epidermal keratinocytes (KCs), mainly in the upper epidermal layers, and in sebocytes of sebaceous glands (Table 1). In contrast, in SGP samples, TSLP was completely or almost completely absent. Importantly, the intensity of TSLP staining (as assessed by Pannoramic Viewer software) was found to be significantly higher in SGR skin compared with SGP skin. However, TSLP expression in SGR skin was significantly lower than that in AD skin (Figure 1a and b). TSLP protein levels were also measured in the stratum corneum by immunocytochemistry and were also found to be significantly elevated in SGR skin compared with SGP skin, but did not reach the level found in AD skin (Figure 1c). Interestingly, quantitative real-time PCR (RT-PCR) analysis detected nearly similar total TSLP mRNA expression in all skin types (SGR, SGP, and AD skin) (Figure 1d).

Linoleic acid induces TSLP expression in KCs

Sebum content, composition of commensal microbiota, and UV radiation are able to influence SGR and SGP skin differently; therefore, the effects of these factors on TSLP production in HaCaT and normal human epidermal keratinocyte cells were analyzed by using RT-PCR and ELISA. As similar TSLP protein levels were detected in hairy scalp (UV-protected) and face (UV-exposed) biopsy samples (Table 1), we did not investigate further the effect of UV.

To study the effect of chitin—a major component of *Demodex folliculorum*, which is part of the normal skin flora in SGR skin—and sebum, HaCaT KCs were treated with chitin (Figure 2a), with supernatant of cultured human SZ95 sebocytes (Zouboulis et al., 1999) (Figure 2b) and with different lipid components of sebum (Figure 2c). After chitin and sebocyte supernatant treatment, induction of TSLP mRNA could be nonsignificantly triggered. Of the used lipid components, palmitic acid, oleic acid, and linoleic acid upregulated TSLP gene expression, but only linoleic acid could elevate it significantly. Furthermore, we showed that linoleic acid induces TSLP mRNA expression in a concentration-dependent manner, reaching its maximum and significantly higher level at 150 μM (Figure 2d and e). On the other hand, the basal TSLP protein levels could not be elevated by any of the aforementioned agents (Figure 2a–c). As sebum components influenced prominently TSLP expression in HaCaT cells, these experiments were repeated in normal human epidermal keratinocytes and similarly linoleic acid could dose-dependently elevate TSLP mRNA levels (Figure 2f and g). No TSLP protein secretion by normal human epidermal keratinocytes could be detected (not shown). It has previously been found in AD skin that barrier damage can also lead to TSLP production by KCs (Mocsai et al., 2014); therefore, transepidermal water loss and skin pH, representing barrier functions, were measured on SGP and SGR skin regions. No differences were detected, indicating that barrier damage is most probably not the cause of distinct TSLP production in SGR and SGP skin (not shown).

SGR skin is characterized by an elevated number of DCs without prominent activation and maturation compared with SGP skin

The significantly higher TSLP level of SGR skin suggested that differences in other immune surveillance factors may also exist. Because DCs are the major target cells of TSLP, CD11c+ dermal myeloid DCs and CD1a+ Langerhans cells were immunolabeled and quantified in SGR and SGP skin samples. IHC revealed that CD11c+ DCs were present in significantly higher numbers (Figure 3a and d) in SGR skin compared with SGP skin and the majority of these cells were characteristically localized near sebaceous glands or the duct of the glands. In AD skin, DC count was higher compared with SGR skin and DCs were found to be diffusely infiltrated in the dermis (Figure 3a and d). In contrast, no significant differences were found between the LC counts of SGP and SGR skin samples (Figure 3g; see Supplementary Figure S1a online).

To further analyze the characteristics of DCs, their classical maturation and/or activation markers CD80, CD83, CD86, and DC-lysosomal associated membrane protein were investigated on the mRNA level. As the classical proinflammatory effect of TSLP is to boost Th2 polarizing DCs in allergic diseases, thymus- and activation-regulated chemokine (TARC) (also known as chemokine [C-C motif] ligand 17 [CCL17]), an atopic eczema-specific, DC-secreted chemokine, and CD83 were also assessed by IHC. Although the number of CD83 positive cells (Figure 3c and f) and mRNA levels of CD80 (Figure 3h), CD83 (Figure 3i), CD86 (Figure 3k), and lysosomal associated membrane protein 3 (CD208) (Figure 3j) could be found in somewhat higher amounts in SGR skin compared with SGP, none of the investigated markers' expression differed significantly, whereas significantly higher numbers of CD83+ cells were detectable in AD samples (Figure 3c and f). TARC was completely absent from both types of healthy skin, but was present in AD samples (Figure 3b and e).