Rosacea as a Disease of Cathelicidins and Skin Innate Immunity

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Rosacea is a common and chronic inflammatory skin disease most frequently seen in groups of genetically related individuals. Although the symptoms of rosacea are heterogeneous, they are all related by the presence of characteristic facial or ocular inflammation involving both the vascular and tissue stroma. Until recently, the pathophysiology of this disease was limited to descriptions of a wide variety of factors that exacerbate or improve disease. Recent molecular studies show a common link between the triggers of rosacea and the cellular response, and these observations suggest that an altered innate immune response is involved in disease pathogenesis. Understanding rosacea as a disorder of innate immunity explains the benefits of current treatments and suggests new therapeutic strategies for alleviating this disease.

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INTRODUCTION

As the phenotypes of rosacea are clinically heterogeneous, rosacea studies were diversely conducted based on the clinical manifestations, histology, and factors exacerbating the skin disorder. From these diverse findings, the pathology of rosacea was thought to be 'unknown' and was expected to be caused by multiple factors. Emotional stress, spicy food, hot beverages, alcohol consumption, high environmental temperatures, sun exposure, and menopause exacerbate the rosacea symptoms such as erythema, rash, and telangiectasia (Crawford *et al.*, 2004; Buechner, 2005). For example, studies have shown increased density of mites in patients with rosacea compared with control patients (Bonnar *et al.*, 1993; Forton and Seys, 1993; Erbagci and Ozgoztasi, 1998), a controversial correlation of *Helicobacter pylori* infection and rosacea (Rebora *et al.*, 1994; Jones *et al.*, 1998; Szlachcic, 2002; Argenziano *et al.*, 2003; Diaz *et al.*, 2003), as well as other varied potential microbial triggers. These findings implied that the external environment would affect rosacea, but is not sufficient or specific to the disease. In the other words, specific intrinsic factors in the host that recognizes and responds to the diverse environmental triggers must be key to understanding the pathogenesis of rosacea.

We recently reported findings of a common aberrant innate immune response in rosacea that when triggered in mice could reproduce characteristic aspects of the human disease (Yamasaki *et al.*, 2007). This article discusses the possible pathophysiology of rosacea through the window of the innate immune system.

ABERRANT CATHELICIDIN EXPRESSION IN ROSACEA SKIN

Strong experimental data from both mice and human tissues have led us to hypothesize that a dysregulation of the innate immune system in patients with rosacea could unify current clinical observations. In innate immunity, the pattern recognition system, which includes the Toll-like receptor (TLR) families, respond to environmental stimuli such as UV, microbes, physical, and chemical trauma. Triggering the innate immune system normally leads to a controlled increase in cytokines and antimicrobial molecules in the skin (Takeda *et al.*, 2003; Meylan *et al.*, 2006). One of these antimicrobial molecules is a peptide known as cathelicidin (Dorschner *et al.*, 2001), the first antimicrobial peptide discovered in mammalian skin (Gallo *et al.*, 1994). Cathelicidin is barely detectable in granular to cornified layers of normal skin, and is greatly induced by the skin wounding and infection to protect the damaged epidermis from microbe invasion (Nizet *et al.*, 2001). Some forms of cathelicidin peptides have a unique capacity to be both vasoactive and proinflammatory (Koczulla *et al.*, 2003; Braff *et al.*, 2005). Therefore, given the potential for a single molecule to affect both of the vascular and inflammatory events that define rosacea, we began an analysis of cathelicidin in affected patients compared with matched controls. Individuals with rosacea expressed abnormally high levels of cathelicidin in epidermis (Yamasaki *et al.*, 2007). Importantly, the cathelicidin peptide forms found in rosacea were not only more

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Abbreviations: CAMP, cathelicidin antimicrobial peptide; KLK, kallikrein; TLR, Toll-like receptor

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abundant but were also of different molecular weights compared with those in normal individuals. These forms of cathelicidin peptides promote and regulate leukocyte chemotaxis (De *et al.*, 2000), angiogenesis (Koczulla *et al.*, 2003), and expression of extracellular matrix components (Gallo *et al.*, 1994), whereas the types most commonly found on normal skin function mostly as antibiotics and have little to no action in inflammation (Murakami *et al.*, 2004; Braff *et al.*, 2005). One of the most common forms of cathelicidin found in rosacea is LL-37 (Yamasaki *et al.*, 2007). This cathelicidin is typically present in neutrophils recruited to infected or injured skin; however, in the case of rosacea patients LL-37 appears to be generated in the epidermis by an abnormal action of serine proteases. When LL-37 is applied in animal models, it was found to be a potent angiogenic factor and resulted in neovascularization in a rabbit model of hind-limb ischemia (Koczulla *et al.*, 2003). Angiogenesis by LL-37 is mediated by formyl peptide receptor-like 1, a G-protein-coupled receptor expressed on endothelial cells (Koczulla *et al.*, 2003). LL-37 also transactivates epidermal growth factor receptor and downstream signaling in epithelial cells (Tjabringa *et al.*, 2003; Tokumaru *et al.*, 2005), and epidermal growth factor receptor signaling induces vascular endothelial growth factor in epidermal keratinocytes (Detmar *et al.*, 1994). Thus, LL-37 induces endothelial cell changes through several signaling pathways, and is a common explanation for vascular effects.

To confirm the importance of these observations in rosacea skin and test the hypothesis that abnormal cathelicidin could induce the signs of rosacea, we injected these peptides or the enzymes that produce cathelicidin into the skin of mice. This rapidly resulted in skin inflammation resembling pathological changes in rosacea (Yamasaki *et al.*, 2007). Combined, these findings indicated that an exacerbated innate immune response induces abnormal cathelicidin, and that this then leads to the clinical findings, therefore confirming our hypothesis.

ROSACEA SKIN INCREASES ACTIVITY OF KALLIKREIN 5, A CATHELICIDIN-PROCESSING SERINE PROTEASE

The presence of the vasoactive and inflammatory cathelicidin peptides in rosacea was subsequently explained by abnormal production of local serine protease kallikrein 5 (KLK5, also know as stratum corneum tryptic enzyme), which processes cathelicidin peptides from a precursor protein in the epidermis (Yamasaki *et al.*, 2006). Entire epidermis of rosacea skin shows increased expression of KLK5 and subsequently high protease activity (Yamasaki *et al.*, 2007). Interestingly, mouse skin lacking lympho-epithelial kazal type-related inhibitor, the intrinsic inhibitor of KLK5, also shows high protease activity and high cathelicidin peptide production (Yamasaki *et al.*, 2007). Similarly, when active human KLK5 was injected into mouse skin, cathelicidin processing increased and was accompanied by skin inflammation. This did not occur if the proteolytic activity of KLK5 was destroyed by heating the enzyme before injection. Combined, these multiple lines of experimental evidence in animal models suggested that the abnormally high protease activity found in rosacea patients results in processing forms of cathelicidin peptides to peptides that induce the characteristic inflammation and vascular changes of this disease.

ALTERED TOLL-LIKE RECEPTOR 2 EXPRESSION IN ROSACEA

A critical question remains as to why individuals prone to rosacea react with high KLK5 and cathelicidin. A potential explanation for this response can be found by understanding that the innate immune system of the skin is programmed to detect microbes, tissue damage such as UV-induced apoptosis, or damage of the extracellular matrix (Chen *et al.*, 2007; Taylor *et al.*, 2007). Among multiple detection systems, TLRs are an abundant and powerful mechanism that widely recognizes microbe derivatives and induces cellular responses such as cytokines and antimicrobial peptides. In the skin of individuals with rosacea, TLR2 was recently found to be more highly expressed compared with that of non-affected individuals (Yamasaki *et al.*, 2011). Increased TLR2 enhances skin susceptibility to specific environmental stimuli and leads to increased cathelicidin production. Cathelicidin transcription in epidermal keratinocytes is also regulated by the active form of vitamin D $1,25(OH)_2D3$ (Schauber *et al.*, 2006). TLR2 stimulation amplifies this by increasing the enzymatic conversion of 25(OH)D3 to active $1,25(OH)_2D3$ (Liu *et al.*, 2006; Schauber *et al.*, 2007). Thus, human epidermal keratinocytes can produce more $1,25(OH)_2D3$ locally by TLR2 stimuli, and this in turn enables the epidermis to produce cathelicidin antimicrobial peptides. KLK5 mRNA transcription is also increased by $1,25(OH)_2D3$ keratinocytes (Morizane *et al.*, 2010). Evidence supporting this conclusion was obtained by genetic overexpression of TLR2 with a TLR2-expressing vector, or testing the response of mice with a knockout of the *TLR2* gene. In these situations, it was found that increasing or stimulating TLR2 increased KLK5, whereas knocking out TLR2 decreased KLK5 (Yamasaki *et al.*, 2011). These findings therefore suggest that the increase of TLR2 in rosacea skin makes the skin of these patients susceptible to microbes and environmental stimuli, therefore resulting in the high cathelicidin and KLK5 expressions, which drive the disease.

Interestingly, TLR2 involvement has also been suggested in other dermatoses resembling rosacea. Glucocorticoid-induced rosacea-like dermatitis, so-called perioral dermatitis, includes erythema, pustules, and papules somewhat similar to that seen in rosacea. Although the precise molecular mechanisms of the steroid-induced dermatitis is not determined, Shibata *et al.* (2009) reported that glucocorticoids increase TLR2 expression in epidermal keratinocytes. They also showed that *Propionibacterium acnes* enhanced glucocorticoid-dependent TLR2 induction, which was abolished by RU486, a glucocorticoid receptor antagonist (Shibata *et al.*, 2009). *P. acnes* is known to activate TLR2 and induce inflammatory cytokines in acne (Kim *et al.*, 2002). Furthermore, the clinical benefits of retinoic acid for rosacea and acne could be partially explained by the ability of retinoic acid to decrease TLR2 expression and function (Liu *et al.*, 2005). Thus, these new findings and accumulated knowledge on rosacea and related dermatoses suggest that TLR2 has a role in disease pathogenesis.

Honoring the memory of Dr Albert Kligman, an article on *Demodex folliculorum*, a mite that lives within sebaceous follicles, was the subject of a recent commentary in this journal (Kligman and Christensen, 2011). As discussed in the article, *Demodex* has been implicated as a trigger of rosacea as histological studies revealed inflammation of the pilosebaceous follicle units, and studies have shown increased density of the mites in patients with rosacea compared with control patients (Bonnar *et al*., 1993; Forton and Seys, 1993; Erbagci and Ozgoztasi, 1998). Lacey *et al*. (2007) isolated *Bacillus oleronius* from *D. folliculorum* and identified the antigens reacting to sera from rosacea individuals but not from control individuals. Extracts of *B. oleronius* stimulate proliferation of mononuclear cells from patients with rosacea, suggesting that individuals with rosacea are exposed to *B. oleronius* from *D. folliculorum*. Interestingly, they identified heat-shock proteins and a lipoprotein in the antigenic molecules of *B. oleronius*. Heat-shock protein and lipoproteins are known to be stimulants for TLR2 (Costa *et al*., 2002; Gobert *et al*., 2004). Therefore, reports of triggers for rosacea from microbes associated with *Demodex* are entirely consistent with the conclusion that an abnormally reactive innate immune system is the basis for the pathophysiology of rosacea.

SUMMARY

Understanding the role of cathelicidin in promotion of inflammation and vascular responses in rosacea provides a molecular insight into this disease. The factors that promote clinical exacerbation of the disease also promote production of critical innate immune molecules that serve to connect molecular observations with the characteristic cellular observations of rosacea (Figure 1). Genetic susceptibility within certain human ethnic groups probably reflects a specific innate immune response programmed within these patients, which makes them more susceptible to certain stimuli. Although much work needs to be done, these new associations give us clues to further our understanding of the mechanisms responsible for the disease and of strategies for the optimal treatment.

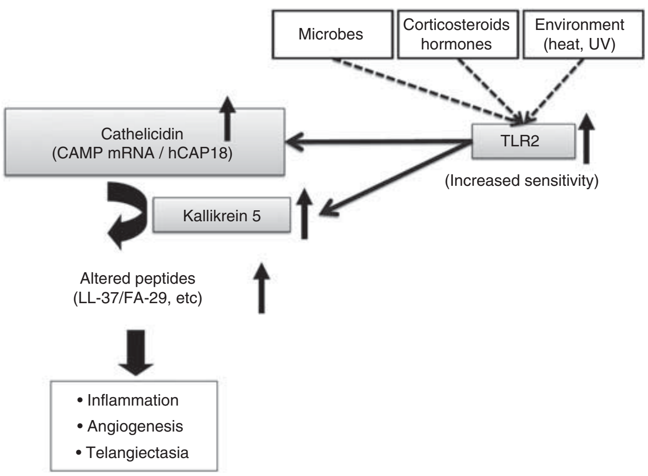

Figure 1. **Molecular mechanisms for the pathogenesis of rosacea.** Rosacea skin is susceptible to environmental changes, altered hormone balance, and microbe challenges because of increased Toll-like receptor 2 (TLR2). The activation of TLR2 then induces an increase in effector molecules: cathelicidin and kallikrein 5 (KLK5). Elevated KLK5 results in generation of active peptides such as LL-37. This peptide stimulates vascular changes and inflammatory cell recruitment. CAMP, cathelicidin antimicrobial peptide.

CONFLICT OF INTEREST

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