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Review article

The role of altered cutaneous immune responses in the induction and persistence of rosacea

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ABSTRACT

Rosacea is a chronic inflammatory skin condition that predominantly affects the skin of the face and the eyes. Several factors are associated with the onset and persistence of the condition, including an altered immune response in the skin and elevated levels of *Demodex* mites. Alterations in the immune response include elevated levels of LL-37 in rosacea skin, increased expression of TLR-2 and increased amounts of vitamin D_3 in epidermal tissue. The combined effect of these changes may make the skin more sensitive to external and internal stimuli. External stimuli that may trigger or sustain rosacea inflammation include exposure to ultraviolet light, while internal factors may include the presence of elevated numbers of *Demodex* mites. These mites may directly stimulate an immune response or release bacteria within the pilosebaceous unit that act as a trigger for inflammation. This review will highlight the changes that occur in the immune response of the skin and describe how *Demodex* mites and associated bacteria may activate this response and lead to the characteristics of rosacea.

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1. Introduction

Rosacea is a chronic cutaneous disorder that primarily affects the central region of the face and is characterized by chronic inflammation and fibrosis [1]. The standard classification system for rosacea describes four distinct clinical subtypes [2]. Erythematotelangiectatic rosacea (ETR) is characterised by flushing and persistent central facial erythema with or without telangiectasia (Fig. 1). Papulopustular rosacea (PPR) is associated

Abbreviations: AMPs, antimicrobial peptides; ETR, erythematotelangiectatic rosacea; hTCEpi, telomerase-immortalized human corneal epithelial cell line; MC, mast cells; KLK5, kallikrein 5; PPR, papulopustular rosacea; PRR, pattern recognition receptors; TLR, toll-like receptor; PAMPs, pathogen-associated molecular patterns; DAMPs, danger-associated molecular patterns.

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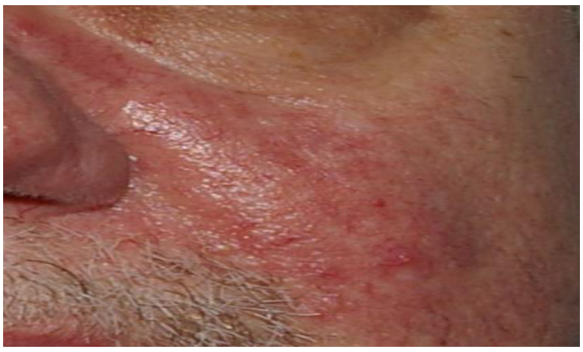

Fig. 1. Erythemic rosacea showing flushing and redness in the centre of the face.

with persistent central facial erythema with transient, central facial papules or pustules or both (Fig. 2). Phymatous rosacea is characterized by skin thickening, irregular surface nodularities and enlargement (Fig. 3). Locations affected include the nose, chin, forehead, cheeks, and ears. Ocular rosacea, displays symptoms such as foreign body sensation in the eye, burning or stinging, dryness, itching, ocular photosensitivity, blurred vision, telangiectasia of the sclera or other parts of the eye, or periorbital oedema (Fig. 4). While four subtypes of rosacea are recognised patients can display a number of subtypes simultaneously e.g. ocular rosacea in erythematotelangiectatic rosacea patients.

The incidence of rosacea in the population may range from less than 1% to 22%, with a higher prevalence amongst fair-skinned individuals of northern European or Celtic ancestry [3]. Females are more likely to suffer the symptoms of rosacea, the onset of which usually occurs between the ages of 30–50 [4]. Males, however are at greater risk of developing the phymatous form of the condition, particularly around the nasal area (rhinophyma) [3].

Although its aetiology remains unclear, the pathophysiology of rosacea appears to be dictated by the complex interaction of a dysfunctional cutaneous-innate immune system and a dysregulated neurovascular system [1,5]. This review will explore some of the key components that contribute to the development of rosacea focusing particularly on the role of the cutaneous-innate immune response, and how certain environmental and microbial stimuli influence this immune response to produce the phenotypic characteristics of rosacea.

1.1. The cutaneous immune response

Within the various epidermal layers of the skin multiple cell-types act synergistically to maintain homeostasis and defend the host against disease [6]. Epidermal keratinocytes are skin cells that

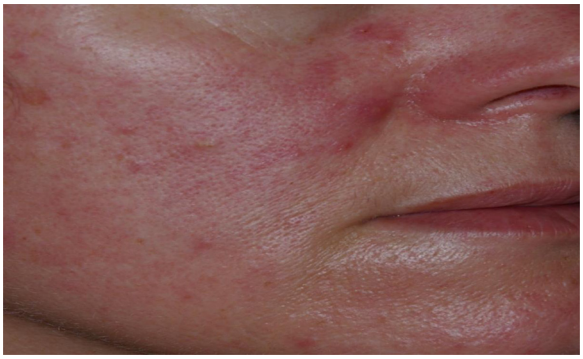

Fig. 2. Papulopustular rosacea showing persistent erythema, teleangiectases, papules and pustules.

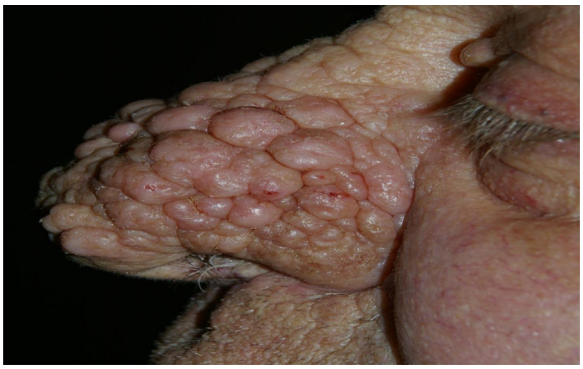

Fig. 3. Phytamous rosacea with proliferation and inflammation of skin surrounding nasal passage.

primarily dictate the structure of the epidermis but also play a role as innate immune cells by detecting pathogens and tissue damage. This process is mediated by pattern recognition receptors (PRRs) which recognise pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs) respectively [6]. In normal skin, triggering an innate immune response through PRRs such as Toll-like receptors (TLRs) normally induces the carefully controlled expression and release of cytokines, chemokines and antimicrobial peptides (AMPs)—effector molecules that mediate a proinflammatory response by recruiting and activating leucocytes [7]. However, individuals with rosacea do not experience the same tightly regulated inflammatory response. Instead, the pathophysiology of rosacea appears to be defined by consistently abnormal innate immune activity which is exemplified by the chronic inflammation associated with the disease [8].

1.2. Cathelicidin/LL37; a major contributor to inflammation in rosacea

Human cathelicidins are multi-functioning proteins that are stored in the granules of neutrophils and in lamellar bodies of keratinocytes [9]. The nascent cathelicidin antimicrobial peptide (*CAMP*) gene product, hCAP18 is secreted as an inactive pro-protein, and in normal skin expression is kept low but dramatically upregulated upon skin-wounding and infection [9]. Enzymatic processing of hCAP18 generates the biologically active peptide LL-37, which can be cleaved further, to positively or negatively modify its antimicrobial activity [10].

LL-37 proteins play a dual role in the innate immune system by functioning as antimicrobials and as mediators of leucocyte recruitment [11,12]. Additional roles for LL-37 involve wound-healing responses including the induction of angiogenesis through binding formyl peptide receptor-like-1 on endothelial cells and stimulating the expression of extracellular matrix components

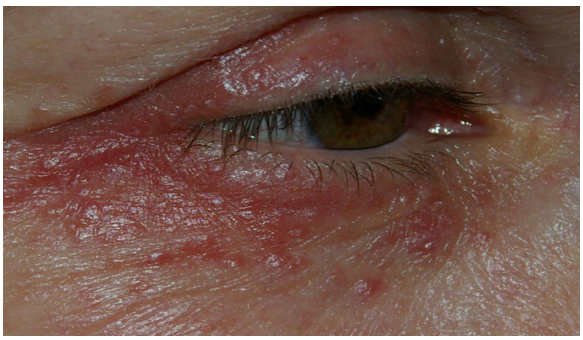

Fig. 4. Ocular rosacea demonstrating eye lid margin inflammation.

[13]. LL-37 promotes keratinocyte migration through the transactivation of epidermal growth factor receptors leading to vascular endothelial growth factor synthesis and, in turn, stimulating vasodilation and angiogenesis [14,15].

Given its influence on proinflammatory- and vaso-activity, LL-37 activity must be strictly regulated however, cathelicidin expression levels are significantly higher in the epidermis of individuals with rosacea than those with normal skin [12]. Thus these peptides have been implicated as key contributors to immune dysregulation in the skin of patients with rosacea [8,12]. Moreover, protein-processing patterns differ and when compared to normal skin, rosacea skin displays an altered cathelicidin profile that includes LL-37 and FA-29 [12]. LL-37 is usually produced in neutrophils activated by injured or infected skin, however aberrant LL-37 production occurs in cells within the epidermis of rosacea skin, giving rise to inflammation, erythema and neovascularization (Fig. 5) [12].

The link between altered LL-37 activity and the inflammation that is characteristic of rosacea skin was demonstrated by Yamasaki et al. who injected mice with exogenous LL-37 and subsequently observed a dose-dependent progression of erythema, vasodilation and neutrophil infiltration [12]. Furthermore, mice deficient in the cathelicidin gene *CAMP*, displayed reduced inflammation in comparison to wild-type mice when treated with a chemical irritant. These results suggest a profound role for the cathelicidin protein, LL-37 in the pathogenesis of rosacea [12].

1.3. Kallikrein-5 (KLK5); the driver of cathelicidin-induced inflammation

The elevated levels of LL-37 in rosacea skin are attributed to increased enzymatic activity of the serine protease kallikrein 5 (KLK5), which cleaves the inactive pro-protein hCAP18 to the biologically active LL-37 peptide [10,12]. Mice deficient in serine protease inhibitors showed increased KLK5 activity and a cathelicidin profile similar to that in rosacea, consisting mainly of GLL-34 (murine LL-37) [12]. In contrast, wild-type mice had no GLL-34 present in the skin. Moreover, when *CAMP*$^{-/-}$ mice were injected with KLK5, the same inflammatory cell infiltrate and erythema observed in wild-type mice did not occur in the cathelicidin$^{-/-}$ mice [12]. Taken together, these observations highlight the importance of KLK5 in orchestrating the inflammation observed in rosacea skin.

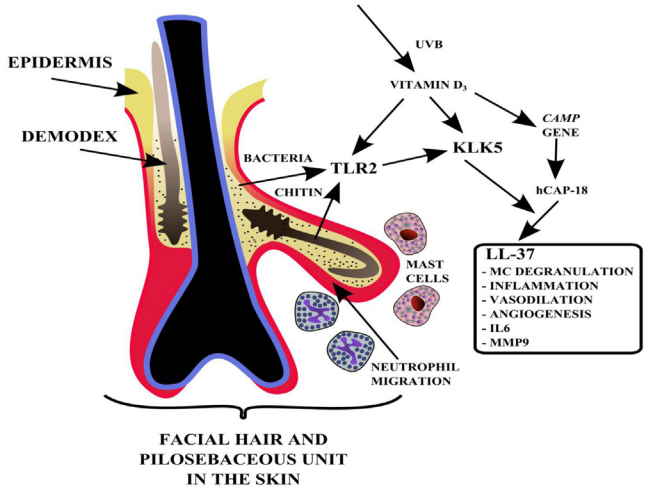

Fig. 5. *Demodex* mites and bacteria in the pilosebaceous unit of the facial skin, along with UV-mediated vitamin D_3 production induce TLR2 activation in keratinocytes, leading the expression of proinflammatory cytokines, chemokines, KLK5 and LL-37. This results in neutrophil and mast cell chemotaxis, and angiogenesis. Neutrophils contribute to inflammation by releasing LL-37 and a range of proinflammatory cytokines as well as MMP3 and MMP9. Mast cells release proinflammatory cytokines, induce KLK5 production and mediate LL-37 activity. The combined effects of these events may result in the formation of pustules and localized inflammation that is characteristic of rosacea.

1.4. Mast cells facilitate the cycle of inflammation in rosacea

Experimental evidence indicates that LL-37 has a profound influence on mast cell (MC) activity by inducing MC chemotaxis, degranulation and proinflammatory cytokine release [16,17]. Muto et al. demonstrated the relationship between MC and LL-37 by injecting MC$^{-/-}$ mice and wild-type mice with exogenous LL-37 [18]. Unlike the wild-type mouse, no inflammation was observed in the MC$^{-/-}$ animal. However, when MC$^{-/-}$ mice were reconstituted with MC and subsequently injected with LL-37, inflammation occurred, thus providing strong evidence for the link between LL-37 and MC activity, and the dependency of each component on the other to induce inflammation. In vitro experiments demonstrated that MC induce KLK5 production by human keratinocytes [18]. Furthermore, exposure of MC to LL-37 induced the release of proinflammatory IL-6 and MMP9 [18] (Fig. 5). Thus MC appear to feed into a cycle of pro-inflammatory activity driven largely by LL-37.

Because of their role in instigating and enhancing inflammation, vasodilation and angiogenesis, the interplay between LL-37 and MC may have critical implications for the pathogenesis of rosacea and are potential therapeutic targets [18].

1.5. Overexpression of TLR2 increases the sensitivity of rosacea skin to external stimuli

Toll-like receptors are key drivers of the innate immune response and their activation through recognition of PAMPs and DAMPs trigger signalling cascades, leading to the controlled release of proinflammatory cytokines, chemokines and AMPs [7]. Yet despite regular TLR activation by external stimuli such as the hugely diverse microbial populations that inhabit all skin types, the chronic inflammation that is typical of rosacea does not appear on normal skin [8,19].

However, TLR2 expression is far greater on the keratinocytes of rosacea skin than on normal skin and even on skin with other inflammatory conditions [19]. TLR2 activation was found to be a significant regulatory factor of increased KLK5 concentrations and protease activity. This TLR2-mediated KLK5 activity appears to be controlled at the post-transcriptional level in a calcium-dependent manner [19]. Consistent with these observations, TLR2$^{-/-}$ mice showed no increase in KLK5 concentrations upon exposure to *Propionibacterium*, the most common microbe on facial skin and an activator of TLR2 [19]. Moreover, *CAMP* expression was abrogated in TLR$^{-/-}$ mice, thereby indicating a role for TLR2 in the induction of cathelicidin genes [19].

1.6. Vitamin D_3 stimulates inflammation in rosacea skin

Numerous studies have established a role for 1,25$(OH)_2$ vitamin D_3 in the cutaneous-innate immune-, and wound-healing response [20,21]. The biologically active form of the vitamin D_3 hormone, 1,25-dihydroxyvitamin D_3 (1,25$(OH)_2D_3$), is produced in the skin upon exposure to UVB light [22]. Vitamin D receptor elements are located in the promoter of the *CAMP* gene and, accordingly 1,25 $(OH)_2D_3$ regulates cathelicidin gene expression in keratinocytes [22,23].

Interestingly, 1,25$(OH)_2D_3$ appears to regulate expression of TLR2 and when human keratinocytes were cultured in the presence of 1,25$(OH)_2D_3$, TLR2 mRNA, but not that of other TLRs, increased

[21]. Furthermore, activation of TLR2, through injury for example, induces the expression of vitamin D receptors and CYP27B1, the enzyme required to activate $1,25(OH)_2D_3$ [21]. $1,25(OH)_2D_3$ also induces KLK5 expression in keratinocytes resulting in increased LL-37 production [22]. Thus, in addition to directly inducing LL-37 production, $1,25(OH)_2D_3$ also increases expression of TLR2 which, through mediating KLK5 activity, elevates levels of LL-37 [19,21].

It is well established that UVB radiation is a key exacerbator of rosacea [8]. This may be explained in part, by increased KLK5 and LL-37 production induced by $1,25(OH)_2D_3$ upon exposure to sunlight [21]. Furthermore, increased vitamin D levels have been identified in the serum of rosacea patients compared to controls, although larger epidemiological studies to verify the link between vitamin D serum-concentrations and the pathogenesis of rosacea were recommended in this study [23].

1.7. Demodex *mites in humans*

Demodex folliculorum mites live in hair follicles or sebaceous glands while *Demodex brevis* mites live individually and are usually located in the meibomian glands. *Demodex* mites occur in all human races, and about 80–90% of the human population is infected, with colonization reaching 100% in elderly people (36). The elevated density of *Demodex* mites in rosacea skin is well established although the precise impact of the mite on the disorder remains controversial [24–26]. Abnormal sebum composition may enable the proliferation of mites in rosacea skin, and lesions may develop as a result of excessive inflammation triggered by *D. folliculorum* in the pilosebaceous unit [26–28].

Several studies have associated increasingly high *D. folliculorum* infestation in rosacea skin with a corresponding increase in pro-inflammatory mediators such as TNF-α, IL-1β, IL-8 and LL-37 [12,26]. *Demodex* mites may induce an inflammatory response through the expression of PAMPs displayed on the mite exoskeleton, or DAMPs caused by epidermal tissue damage when the mites feed on epithelial cells [29]. Chitin, a polysaccharide contained in the exoskeleton of insects, is known to activate TLR2 on keratinocytes [30] and a role for NLRP3 in the pathogenesis of rosacea was recently reported by Casas et al. who observed increased expression of Nalp3, pro-caspase-1, pro-IL-1β and IL-1β [26]. In this study, individuals with PPR were found to have higher levels of IL-8 than those with ETR [26]. In addition to inducing angiogenesis, IL-8 promotes neutrophil chemotaxis and these cells contribute to the development of inflammatory pustules characteristic of PPR [31].

Due to the fact that rosacea correlates with the increased abundance of *Demodex* mites and patients respond well to antibiotics, it has been speculated that *D. folliculorum* mites may be a reservoir of micro-organisms that contribute to the development of the disease. *Demodex* mites lack an anus and the undigested remains are kept inside the mites, thus providing a micro-environment for a broad range of bacteria in the digestive system [36]. The internal contents may be released upon death of the mite. In this way *Demodex* mites may bring bacteria deep into the skin where an immune reaction is triggered. This may explain the host's inability to effectively eradicate these bacterial infections since the micro-organisms are constantly passively transferred by motile *Demodex* parasites. While the mites themselves evade the immune system, their presence may result in a permanent exposure of human immune system to bacterial antigens, causing a chronic inflammatory condition.

1.8. The potential role of bacteria in rosacea

The effectiveness of antibiotics such as tetracycline as a treatment for reducing inflammation in rosacea indicates that inflammatory processes may be driven by bacteria, since tetracycline does not kill *Demodex* mites [33]. There are conflicting opinions about whether the reduction in inflammation is due to the anti-inflammatory properties or anti-microbial properties of antibiotics [34,35]. Nevertheless, current data suggests that bacteria may indeed have a potential role in the pathogenesis of rosacea [35,36].

Whitfeld et al. reported a role for *Staphylococcus epidermidis* in the development of pustules [37]. *S. epidermidis* strains inhabiting rosacea skin appear to differ from those in normal skin, and when grown at 37 °C these strains produced an altered protein profile compared to those grown at 30 °C [35]. It was suggested that the slightly elevated temperature in the facial skin of rosacea patients may influence the strains of bacteria colonising the skin, hence the proteins they produce [35].

Demodex mites contain a large and varied population of bacteria [32] and when the mites die, bacterial antigens may be released into the pilosebaceous unit, triggering a localised pro-inflammatory response [33]. Since *Demodex* density is increased in rosacea, the bacterial load may also be elevated and these high levels of antigens could aggravate an already aberrant immune response [33,38]. This may explain why inflammation in cases of papulo-pustular rosacea is often centred on the pilosebaceous unit where the *Demodex* mites are located.

Analysis of the microbiota of *Demodex* mites obtained from the skin of individuals with ETR, PPR and normal skin, showed that microbial diversity differed according to the status of the host's skin condition [32]. It has been suggested that *Demodex*-associated microbiota may be influenced by the different sebum composition in rosacea skin [27,32]. Recent research points to a role for the *Demodex*-related bacteria *Bacillus oleronius*, in stimulating proinflammatory responses in PPR, ETR and OR [33,38,39]. *B. oleronius* was isolated from a *D. folliculorum* mite extracted from the face of a patient with papulopustular rosacea [33] and this bacterium was sensitive to tetracycline, doxycycline and minocycline, commonly successfully used in the treatment of rosacea. Of specific interest were *B. oleronius*-derived 62- and 83 kDa proteins as these seemed to trigger significant immunoreactivity in the serum of individuals with rosacea when compared to controls [33,38,39]. Exposure to *B. oleronius* proteins induced high rates of neutrophil chemotaxis and a neutrophil-mediated proinflammatory response involving IL-8, MMP9, TNF-α and cathelicidins [40].

The potential role of *B. oleronius* proteins in the development of corneal ulcers was recently investigated by exposing corneal epithelial cells (hTCEpi) to *Bacillus* proteins and this resulted in the upregulation of several pro-inflammatory cytokines including IL-6, IL-8, IL-1β and TNF-α [41]. In a separate study using the same cell line, significant upregulation of MMP3 and MMP9 was observed by cells exposed to *B. oleronius*, thus leading to a possible explanation for the corneal ulcer formation and corneal scarring in individuals suffering from ocular rosacea [42].

1.9. Therapeutic strategies for rosacea

A number of treatments for rosacea currently exist however these tend to treat distinct symptoms rather than the underlying cause(s) [43]. Topical medications such as azelaic acid and metronidazole have shown effectiveness in the treatment of PPR by virtue of their anti-inflammatory and antimicrobial properties [43]. Oral tetracycline antibiotics have shown success in treating rosacea although it is unclear whether this is because of its antimicrobial properties, or due to its ability to inhibit protease (e.g. KLK5) and MMP activity, and thus reduce inflammation [34,43,44]. Since tetracycline antibiotics are inactive against *Demodex*, it is possible that this drug may be acting on a bacterial element thereby reducing the inflammation in rosacea skin. On the other hand, oral treatment with a sub-antimicrobial dose of

doxycycline, given once daily in 40 mg capsules (30 mg immediate release and 10 mg delayed-release beads) has shown to be as effective as a 100 mg (antimicrobial) dose of doxycycline in reducing inflammation in individuals with rosacea and this course of therapy is now approved by the US FDA for the treatment of rosacea [43,45].

Tea-tree oil (TTO) is a known acaricide and has demonstrated positive effects in reducing the *Demodex* infestation and inflammation in patients with blepharitis [46]. Ivermectin is a broad spectrum antiparasitic drug which also possesses some anti-inflammatory properties [47]. More recently, the use of topical ivermectin (1%) was shown to be effective in reducing the symptoms of PPR [48]. Oral ivermectin has had successful clinical outcomes in treating *Demodex* infestations in patients presenting with blepharitis, resulting in reduced *Demodex* density and improved clinical symptoms [49]. In this study, and others [50], the use of systemic ivermectin did not completely eradicate *Demodex* mites. However, the correlation between the observed reduction of clinical symptoms and reduced *Demodex* infestation in patients treated with ivermectin strengthens the argument that *Demodex* mites play a significant role in the manifestations of OR and related symptoms [49]. The authors of this study proposed the use of ivermectin in conjunction with other therapies to challenge *Demodex* infestation which may be associated with the symptoms of blepharitis. Indeed, combination therapy involving ivermectin and metronidazole has shown excellent clinical outcomes in treating individuals with facial rosacea and blepharitis [50]. A comparison of individuals treated with a combination of metronidazole and ivermectin rather than with ivermectin in isolation, showed a greater reduction in the *Demodex* population in individuals treated with the combined therapy than with ivermectin alone. The anti-inflammatory properties of metronidazole are thought to reduce the symptoms of inflammation that may be caused by *Demodex*-associated antigens and it has been suggested that metronidazole also has acricidal effects, which may complement the anti-*Demodex* activity of ivermectin [50].

2. Conclusion

Many factors may influence the appearance and progression of rosacea. The altered immune response in the skin may make it more sensitive to stimuli that under normal conditions do not provoke a response. The altered sebum content on rosacea skin may favour the increased density of *Demodex* mites which may be stimulatory. Alternatively the mites may carry bacteria which are released upon their death within the pilosebaceous unit and which then stimulate the immune response. A better understanding of the complex relationship between the various factors that influence the onset, persistence and progression of rosacea, may facilitate the development of novel therapies that target the cellular and molecular mechanisms responsible for the pathogenesis of this disfiguring disorder.

Conflicts of interest

The authors have no conflicts of interest to declare.

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