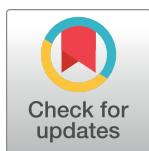PEARLS

Innate antimicrobial immunity in the skin: A protective barrier against bacteria, viruses, and fungi

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Innate immunity is an essential defense against pathogens

The epidermis, the outermost layer of the skin, is a physical barrier against pathogens. However, breach of the skin barrier through wounding introduces a myriad of microbes to the site of injury. Upon disturbance of the epidermal barrier, the innate immune system and its effectors play a key role in protecting humans against cutaneous and systemic infection [1]. Major constituents of the innate immune system include phagocytic cells, such as macrophages, neutrophils, and dendritic cells, as well as innate leukocytes, such as natural killer (NK) cells, mast cells, basophils, and eosinophils. In addition, epidermal keratinocytes act as active innate immune cells. In response to sensing pathogen-associated molecular patterns (PAMPs) expressed by microbes and host danger molecules, innate immune receptors present on keratinocytes become activated, causing release of inflammatory cytokines and host antimicrobial molecules [2, 3].

Recognition of pathogens

The first step of any immune response is recognition of potential pathogens. Germline-encoded pattern recognition receptors (PRRs) recognize PAMPs present on microbes and damaged-associated molecular patterns (DAMPs) on host cells (Fig 1) [4]. The four primary groups of human PRRs are toll-like receptors (TLRs), nucleotide-binding oligomerization domain-like receptors (NLRs), retinoic acid-inducible gene 1 (RIG-I)-like helicase receptors (RLRs) and c-type lectin receptors (CLRs) [4]. Signaling through PRRs has long been known to be essential for activation of the innate immune response. For example, stimulation of TLR2 increases the immune response to pathogens and helps rescue the inflammatory response of immunosuppressed patients with sepsis [5]. Although PRRs are not as specific as immune effectors of the adaptive immune system, different PRRs have evolved to recognize different molecular patterns [6]. For example, TLR2, TLR6, and nucleotide-binding oligomerization domain-containing protein 2 (NOD2) appear to play an important role in host defense against staphylococcal aureus, whereas TLRs 2, 3, 7, 8, and 9 have been found to be activated by many viruses, including members of the herpesviruses, papillomaviruses, and poxviruses [7, 8]. CLRs and TLRs 2, 4, and 9 are thought to be primary receptors involved in recognition of fungal pathogens such as *Candida albicans*, and there are reports of specific PRR deficiencies in patients with chronic mucocutaneous infections [9, 10].
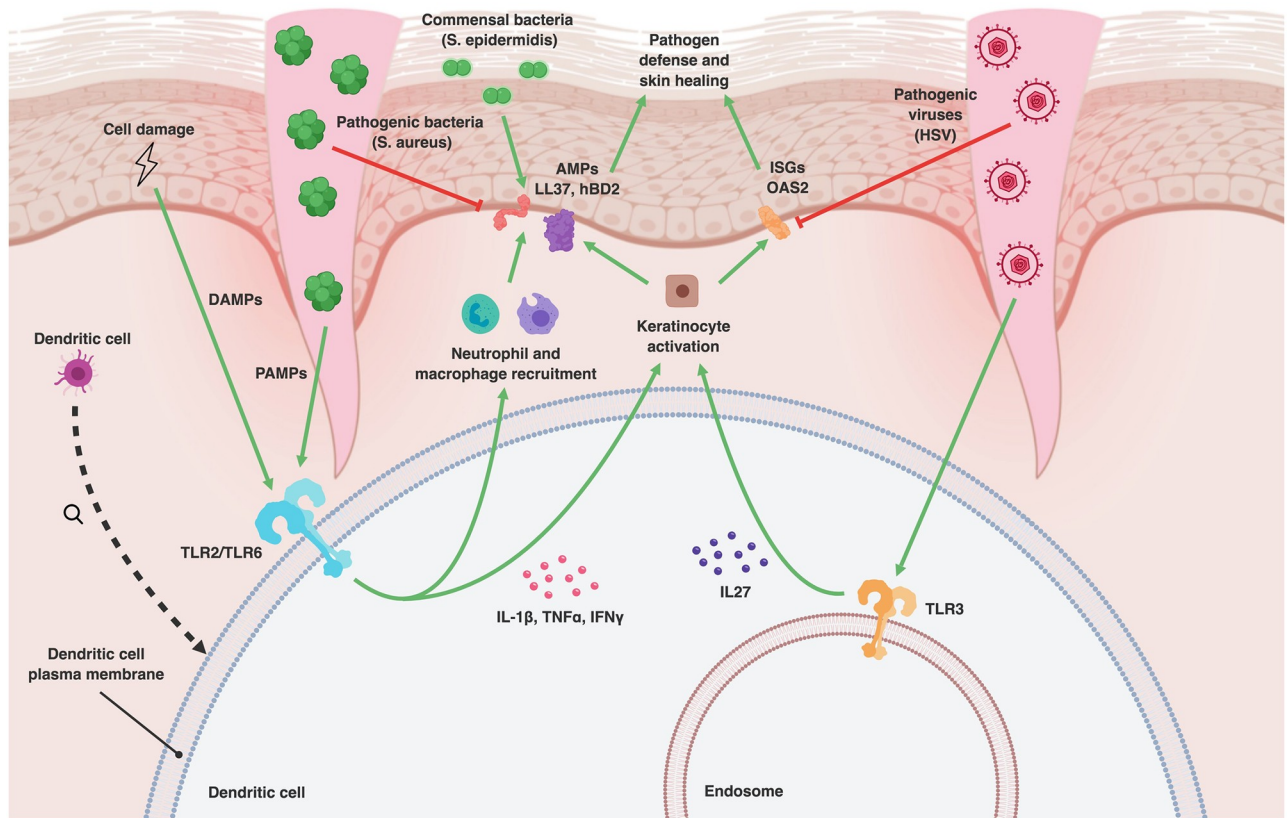
Fig 1. Antimicrobial and antiviral signaling in the skin. * Skin injury allows pathogenic bacteria and viruses to penetrate the epidermis. PAMPs and DAMPs are recognized by TLRs, which induce the projection of pro-inflammatory cytokines by dendritic cells. Examples of pro-inflammatory cytokines include IL-1β, TNFα, and IFNγ. Inflammatory cytokines recruit neutrophils and macrophages to the site of injury and promote production of AMPs by these inflammatory cells and also by keratinocytes. The AMPs produced include cathelicidins (LL-37) and defensins (hBD2). Some TLRs, such as TLR3, recognize viral components. IL-27 is produced in response to TLR3 activation and induces translation of anti-viral ISGs, such as OAS2, by keratinocytes. AMPs and ISGs are important effector molecules for pathogen defense and skin healing. Commensal bacteria, such as *Staphylococcus epidermidis*, promote AMP production. Pathogenic bacteria, such as *S. aureus*, inhibit the production of AMPs; pathogenic viruses, such as HSV, inhibit ISG production. *Created with BioRender. AMP, antimicrobial protein; DAMP, damage-associated molecular pattern; hBD2, human β-defensin-2; HSV, herpes simplex virus; IL, interleukin; IFN, interferon; ISG, interferon-stimulated gene; OAS, oligoadenylate synthetase; PAMP, pathogen-associated molecular pattern; TLR, toll-like receptor; TNF, tumor necrosis factor.

The inflammatory cascade

Activation of PRRs leads to initiation of the inflammatory cascade. Soon after hemostasis, adhesion molecules are expressed in response to pro-inflammatory cytokines, such as interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and interferon-γ (IFNγ) [11]. Polymorphic neutrophils are recruited by CXC chemokines containing asparagine-leucine-arginine (ELR) motifs, such as IL-8, which transmigrate across capillary walls and interact with adhesion molecules [12]. Neutrophils begin phagocytosis of pathogens and tissue debridement. Later during inflammatory cascade, macrophages are the predominant immune cell type. Macrophages of the pro-inflammatory phenotype (M1) continue phagocytosis as well as amplify the inflammatory response. Anti-inflammatory cytokines, such as transforming growth factor β (TGFβ), cause inflammatory M1 macrophages to shift to an anti-inflammatory phenotype (M2), which promotes wound repair and closure [12].

Endogenous antibacterial and antiviral proteins help the innate immune system fight pathogenic organisms

Antimicrobial proteins (AMPs) are produced by keratinocytes, infiltrating immune cells, and skin commensal organisms and provide antibiotic-like protection for the skin. AMPs are directly bactericidal through cell lysis, with a preference for prokaryotic cell membranes [13]. Other antimicrobial mechanisms include inhibition of bacteria protein and DNA synthesis. Antifungal mechanism of AMPs involve disruption of the fungal mitochondrial membrane [13]. Two families of AMPs, cathelicidins and defensins, are illustrative of the potent activity of these proteins. Cathelicidins and defensins provide broad-spectrum protection against gram-positive and gram-negative bacteria [14]. For example, the human cathelicidin LL-37 has potent direct antibacterial activity against bacteria such as Group A *Streptococcus* (GAS), and mice deficient in cathelicidins have been shown to have higher susceptibility to GAS infection [15]. Decreased expression of human cathelicidin LL-37 and human β-defensin-2 (hBD-2) in lesions of atopic dermatitis compared to amplified expression in psoriatic lesions may help account for the increased susceptibility to secondary bacterial infection that is seen in patients with atopic dermatitis but not psoriasis [16]. Some defensins, including hBD1, are expressed constitutively in epithelial cells [14], whereas others, such as hBD2, are only constitutively expressed at very low levels, with a dramatic increase in their production during inflammation [17]. One AMP, dermcidin, is released by sweat glands and displays broad antimicrobial activity, demonstrating a role for sweat in microbial protection [18]. In addition to direct activity against pathogens, AMPs also have immunomodulatory activity. For example, LL-37 induces chemotaxis of neutrophils, monocytes, mast cells, and T cells. LL-37 levels are also noted to be dramatically lower in chronic ulcers than in wounds that undergo normal healing, which highlights the importance of this AMP in wound healing, in addition to innate immunity [19]. hBD2 displays chemokine-like activity to attract dendritic cells and memory T cells and also promotes histamine release by mast cells [14]. Finally, regenerating islet-derived protein 3A (REG3A), an AMP present in the gut as well as the skin, promotes keratinocyte proliferation, suggesting a role for AMPs in wound healing as well as antimicrobial defense [20].

PRRs also recognize viral components, leading to transcription of antiviral interferon-stimulated genes (ISGs). One example of an ISG is oligoadenylate synthetase (OAS). OAS identifies viruses through binding of viral double stranded RNA (dsRNA) and activation of an intracellular latent RNase (RNase L), which leads to the degradation of viral RNA [21]. OAS proteins also function through an RNase L–independent mechanism in which proteins that are released from virus-infected cells act extracellularly as an antiviral agent through paracrine signaling [22]. Recent discoveries have highlighted the importance of IL-27 signaling in wound healing and ISG responses [23]. ISGs become strongly up-regulated in epidermal keratinocytes following stimulation with recombinant IL-27, and mice lacking the IL-27 receptor have been found to have delayed wound healing.

How pathogenic microbes counteract immune defenses

Staphylococcus aureus is the most common cause of bacterial skin infections. The mechanisms by which it evades eradication by the innate immune system are representative of the strategies employed by other microbes to counteract immune defenses. For example, *S. aureus* releases staphylococcal superantigen-like proteins and toxins that prevent neutrophil migration and cause neutrophil lysis [24]. A second evasion strategy involves the release of membrane vesicles that contain factors that inactivate the complement system. [25]. One such factor is Staphopain A, which cleaves elastin and inactivates C-X-C motif chemokine receptor 2 (CXCR2) and

C5b-complement [26]. *S. aureus* also produces a metalloproteinase called aureolysin that cleaves and inactivates the AMP LL-37 [27]. Another protease, staphylokinase, undergoes complex formation with human defenses, leading to their inactivation [28]. *S. aureus* is also able to alter its hydrophobicity through production of a surface protein, iron-regulated surface determinant protein A (IsdA), resulting in resistance to hBD2 and LL-37 [29].

Viruses that affect the skin also have virulence factors that aid in evasion of the innate immune system. Production of IFN-α and IFN-β is decreased in skin samples infected with herpes simplex virus 2 (HSV2) [30]. The action of OAS2 is prevented by secretion of Us11 protein by HSV1 [31]. Poxviruses, particularly vaccinia virus, have become particularly adept at evading host immunity through the production of peptides that block activation of TLRs [32] Other viruses and pathogenic microbes employ similar mechanisms for immune evasion.

Commensal organisms promote eradication of pathogenic bacteria and viruses and encourage healing

Previous studies have demonstrated that a synergistic relationship between the human host and the commensal skin microbiome promotes successful wound healing and overall health. Skin commensal microbes, such as *S. epidermidis*, produce AMPs that act alongside endogenous AMPs produced by human keratinocytes to provide antibiotic-like protection for the skin; *S. epidermidis* also enhances the production of AMPs by keratinocytes [2]. One study identified a small molecule produced by *S. epidermidis* that activates TLR2 signaling and induces AMP production by keratinocytes [33]. Other peptides produced by *S. epidermidis*, such as a group of phenol-soluble modulins, display direct antimicrobial action against pathogenic bacteria, including *S. aureus* [34]. *S. epidermidis* can even prevent uncontrolled inflammation, a hallmark of chronic wounds [33]. Commensal bacteria also modulate antiviral immunity. Lipoteichoic acid, a cell-wall component of gram-positive bacteria, increases mast cell activity against vaccina viruses [35].

Too much of a good thing: Immune hyperactivation and microbial superinfection

Excess inflammation underlies multiple common skin pathologies and is detrimental to skin healing. One of the most common skin conditions, acne vulgaris, is characterized by excess production of pro-inflammatory cytokines and dermal inflammation. Inflammation of the skin may even precede infection with *Propionibacterium acnes* [36]. Rosacea is also marked by excess inflammation; TLR2 sensitivity is increased in lesional keratinocytes [37]. Similarly, REG3A, a peptide that promotes wound reepithelialization, inhibits keratinocyte terminal differentiation and promotes keratinocyte hyperproliferation in psoriatic skin [20]. In addition to contributing to the pathogenesis of skin disease, excess inflammation impedes healing by preventing progression into the proliferative phase of wound closure. Excess inflammation is particularly prevalent in diabetic ulcers and venous ulcers.

Conclusion

The innate immune system is integral to the prevention of skin infection and eradication of pathogenic bacteria and plays an essential role in skin healing. Recognition of bacteria and viruses initiates the inflammatory cascade involving the release of cytokines, recruitment of immune cells, and production of AMPs and ISGs. AMPs and ISGs represent one of the most important and robust immune mechanisms in the skin. However, pathogenic bacteria—such as *S. aureus*—and cutaneous viruses have evolved mechanisms to counteract innate immune

mechanisms. Commensal skin bacteria assist the innate immune system with eradication of pathogens through production of AMPs and by enhancing the activity of innate immune cells. Finally, despite the importance of innate immunity, excess immune activation underlies some cutaneous diseases and is detrimental to wound healing.

References

1. Singer AJ, Clark RA. Cutaneous wound healing. The New England journal of medicine. 1999; 341 (10):738–46. Epub 1999/09/02. https://doi.org/10.1056/NEJM199909023411006 PMID: 10471461.

2. Gallo RL, Nakatsuji T. Microbial symbiosis with the innate immune defense system of the skin. The Journal of investigative dermatology. 2011; 131(10):1974–80. Epub 2011/06/24. https://doi.org/10.1038/jid.2011.182 PMID: 21697881.

3. Nelson AM, Reddy SK, Ratliff TS, Hossain MZ, Katseff AS, Zhu AS, et al. dsRNA Released by Tissue Damage Activates TLR3 to Drive Skin Regeneration. Cell stem cell. 2015; 17(2):139–51. Epub 2015/08/09. https://doi.org/10.1016/j.stem.2015.07.008 PMID: 26253200.

4. Takeuchi O, Akira S. Pattern recognition receptors and inflammation. Cell. 2010; 140(6):805–20. Epub 2010/03/23. https://doi.org/10.1016/j.cell.2010.01.022 PMID: 20303872.

5. Raby AC, Holst B, Le Bouder E, Diaz C, Ferran E, Conraux L, et al. Targeting the TLR co-receptor CD14 with TLR2-derived peptides modulates immune responses to pathogens. Science translational medicine. 2013; 5(185):185ra64. Epub 2013/05/17. https://doi.org/10.1126/scitranslmed.3005544 PMID: 23677593.

6. de Koning HD, Simon A, Zeeuwen PL, Schalkwijk J. Pattern recognition receptors in infectious skin diseases. Microbes and infection. 2012; 14(11):881–93. Epub 2012/04/21. https://doi.org/10.1016/j.micinf.2012.03.004 PMID: 22516809.

7. Krishna S, Miller LS. Innate and adaptive immune responses against Staphylococcus aureus skin infections. Seminars in immunopathology. 2012; 34(2):261–80. Epub 2011/11/08. https://doi.org/10.1007/s00281-011-0292-6 PMID: 22057887.

8. Kawamura T, Ogawa Y, Aoki R, Shimada S. Innate and intrinsic antiviral immunity in skin. Journal of dermatological science. 2014; 75(3):159–66. Epub 2014/06/15. https://doi.org/10.1016/j.jdermsci.2014.05.004 PMID: 24928148.

9. Plato A, Hardison SE, Brown GD. Pattern recognition receptors in antifungal immunity. Seminars in immunopathology. 2015; 37(2):97–106. Epub 2014/11/26. https://doi.org/10.1007/s00281-014-0462-4 PMID: 25420452.

10. Ferwerda B, Ferwerda G, Plantinga TS, Willment JA, van Spriel AB, Venselaar H, et al. Human dectin-1 deficiency and mucocutaneous fungal infections. The New England journal of medicine. 2009; 361 (18):1760–7. Epub 2009/10/30. https://doi.org/10.1056/NEJMoa0901053 PMID: 19864674.

11. Eming SA, Krieg T, Davidson JM. Inflammation in wound repair: molecular and cellular mechanisms. The Journal of investigative dermatology. 2007; 127(3):514–25. Epub 2007/02/15. https://doi.org/10.1038/sj.jid.5700701 PMID: 17299434.

12. MacLeod AS, Mansbridge JN. The Innate Immune System in Acute and Chronic Wounds. Advances in wound care. 2016; 5(2):65–78. Epub 2016/02/11. https://doi.org/10.1089/wound.2014.0608 PMID: 26862464.

13. Agerberth B, Gudmundsson GH. Host antimicrobial defence peptides in human disease. Current topics in microbiology and immunology. 2006; 306:67–90. Epub 2006/08/17. PMID: 16909918.

14. Braff MH, Gallo RL. Antimicrobial peptides: an essential component of the skin defensive barrier. Current topics in microbiology and immunology. 2006; 306:91–110. Epub 2006/08/17. PMID: 16909919.

15. Dorschner RA, Pestonjamasp VK, Tamakuwala S, Ohtake T, Rudisill J, Nizet V, et al. Cutaneous injury induces the release of cathelicidin anti-microbial peptides active against group A Streptococcus. The Journal of investigative dermatology. 2001; 117(1):91–7. Epub 2001/07/10. https://doi.org/10.1046/j.1523-1747.2001.01340.x PMID: 11442754.

16. Ong PY, Ohtake T, Brandt C, Strickland I, Boguniewicz M, Ganz T, et al. Endogenous antimicrobial peptides and skin infections in atopic dermatitis. The New England journal of medicine. 2002; 347 (15):1151–60. Epub 2002/10/11. https://doi.org/10.1056/NEJMoa021481 PMID: 12374875.

17. Bals R, Wang X, Wu Z, Freeman T, Bafna V, Zasloff M, et al. Human beta-defensin 2 is a salt-sensitive peptide antibiotic expressed in human lung. The Journal of clinical investigation. 1998; 102(5):874–80. Epub 1998/09/03. https://doi.org/10.1172/JCI2410 PMID: 9727055.

18. Schittek B, Hipfel R, Sauer B, Bauer J, Kalbacher H, Stevanovic S, et al. Dermcidin: a novel human antibiotic peptide secreted by sweat glands. Nature immunology. 2001; 2(12):1133–7. Epub 2001/11/06. https://doi.org/10.1038/ni732 PMID: 11694882.

19. Heilborn JD, Nilsson MF, Kratz G, Weber G, Sorensen O, Borregaard N, et al. The cathelicidin anti-microbial peptide LL-37 is involved in re-epithelialization of human skin wounds and is lacking in chronic ulcer epithelium. The Journal of investigative dermatology. 2003; 120(3):379–89. Epub 2003/02/27. https://doi.org/10.1046/j.1523-1747.2003.12069.x PMID: 12603850.

20. Lai Y, Li D, Li C, Muehleisen B, Radek KA, Park HJ, et al. The antimicrobial protein REG3A regulates keratinocyte proliferation and differentiation after skin injury. Immunity. 2012; 37(1):74–84. Epub 2012/06/26. https://doi.org/10.1016/j.immuni.2012.04.010 PMID: 22727489.

21. Kristiansen H, Gad HH, Eskildsen-Larsen S, Despres P, Hartmann R. The oligoadenylate synthetase family: an ancient protein family with multiple antiviral activities. Journal of interferon & cytokine research: the official journal of the International Society for Interferon and Cytokine Research. 2011; 31 (1):41–7. Epub 2010/12/15. https://doi.org/10.1089/jir.2010.0107 PMID: 21142819.

22. Kristiansen H, Scherer CA, McVean M, Iadonato SP, Vends S, Thavachelvam K, et al. Extracellular 2'-5' oligoadenylate synthetase stimulates RNase L-independent antiviral activity: a novel mechanism of virus-induced innate immunity. Journal of virology. 2010; 84(22):11898–904. Epub 2010/09/17. https://doi.org/10.1128/JVI.01003-10 PMID: 20844035.

23. Yang B, Suwanpradid J, Sanchez-Lagunes R, Choi HW, Hoang P, Wang D, et al. IL-27 Facilitates Skin Wound Healing through Induction of Epidermal Proliferation and Host Defense. The Journal of investigative dermatology. 2017; 137(5):1166–75. Epub 2017/01/31. https://doi.org/10.1016/j.jid.2017.01.010 PMID: 28132857.

24. Zecconi A, Scali F. Staphylococcus aureus virulence factors in evasion from innate immune defenses in human and animal diseases. Immunology letters. 2013; 150(1–2):12–22. Epub 2013/02/05. https://doi.org/10.1016/j.imlet.2013.01.004 PMID: 23376548.

25. Lee EY, Choi DY, Kim DK, Kim JW, Park JO, Kim S, et al. Gram-positive bacteria produce membrane vesicles: proteomics-based characterization of Staphylococcus aureus-derived membrane vesicles. Proteomics. 2009; 9(24):5425–36. Epub 2009/10/17. https://doi.org/10.1002/pmic.200900338 PMID: 19834908.

26. Jusko M, Potempa J, Kantyka T, Bielecka E, Miller HK, Kalinska M, et al. Staphylococcal proteases aid in evasion of the human complement system. Journal of innate immunity. 2014; 6(1):31–46. Epub 2013/07/11. https://doi.org/10.1159/000351458 PMID: 23838186.

27. Sieprawska-Lupa M, Mydel P, Krawczyk K, Wojcik K, Puklo M, Lupa B, et al. Degradation of human antimicrobial peptide LL-37 by Staphylococcus aureus-derived proteinases. Antimicrobial agents and chemotherapy. 2004; 48(12):4673–9. Epub 2004/11/25. https://doi.org/10.1128/AAC.48.12.4673-4679.2004 PMID: 15561843.

28. Jin T, Bokarewa M, Foster T, Mitchell J, Higgins J, Tarkowski A. Staphylococcus aureus resists human defensins by production of staphylokinase, a novel bacterial evasion mechanism. Journal of immunology (Baltimore, Md: 1950). 2004; 172(2):1169–76. Epub 2004/01/07. PMID: 14707093.

29. Clarke SR, Mohamed R, Bian L, Routh AF, Kokai-Kun JF, Mond JJ, et al. The Staphylococcus aureus surface protein IsdA mediates resistance to innate defenses of human skin. Cell host & microbe. 2007; 1(3):199–212. Epub 2007/11/17. https://doi.org/10.1016/j.chom.2007.04.005 PMID: 18005699.

30. Peng T, Zhu J, Klock A, Phasouk K, Huang ML, Koelle DM, et al. Evasion of the mucosal innate immune system by herpes simplex virus type 2. Journal of virology. 2009; 83(23):12559–68. Epub 2009/10/02. https://doi.org/10.1128/JVI.00939-09 PMID: 19793807.

31. Sanchez R, Mohr I. Inhibition of cellular 2'-5' oligoadenylate synthetase by the herpes simplex virus type 1 Us11 protein. Journal of virology. 2007; 81(7):3455–64. Epub 2007/01/19. https://doi.org/10.1128/JVI.02520-06 PMID: 17229694.

32. Brady G, Bowie AG. Innate immune activation of NFkappaB and its antagonism by poxviruses. Cytokine & growth factor reviews. 2014; 25(5):611–20. Epub 2014/08/02. https://doi.org/10.1016/j.cytogfr.2014.07.004 PMID: 25081317.

33. Lai Y, Di Nardo A, Nakatsuji T, Leichtle A, Yang Y, Cogen AL, et al. Commensal bacteria regulate Toll-like receptor 3-dependent inflammation after skin injury. Nature medicine. 2009; 15(12):1377–82. Epub 2009/12/08. https://doi.org/10.1038/nm.2062 PMID: 19966777.

34. Cogen AL, Yamasaki K, Sanchez KM, Dorschner RA, Lai Y, MacLeod DT, et al. Selective antimicrobial action is provided by phenol-soluble modulins derived from Staphylococcus epidermidis, a normal resident of the skin. The Journal of investigative dermatology. 2010; 130(1):192–200. Epub 2009/08/28. https://doi.org/10.1038/jid.2009.243 PMID: 19710683.

35. Wang Z, MacLeod DT, Di Nardo A. Commensal bacteria lipoteichoic acid increases skin mast cell antimicrobial activity against vaccinia viruses. Journal of immunology (Baltimore, Md: 1950). 2012; 189 (4):1551–8. Epub 2012/07/10. https://doi.org/10.4049/jimmunol.1200471 PMID: 22772452.

36. Dainichi T, Hanakawa S, Kabashima K. Classification of inflammatory skin diseases: a proposal based on the disorders of the three-layered defense systems, barrier, innate immunity and acquired immunity.

Journal of dermatological science. 2014; 76(2):81–9. Epub 2014/09/23. https://doi.org/10.1016/j.jdermsci.2014.08.010 PMID: 25242498.

37. Yamasaki K, Kanada K, Macleod DT, Borkowski AW, Morizane S, Nakatsuji T, et al. TLR2 expression is increased in rosacea and stimulates enhanced serine protease production by keratinocytes. The Journal of investigative dermatology. 2011; 131(3):688–97. Epub 2010/11/26. https://doi.org/10.1038/jid.2010.351 PMID: 21107351.