

COMMENTARY

other autoantibody-mediated autoimmune disorders. Although further research is required, FcRn inhibition represents a significant step forward in the management of pemphigus.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

REFERENCES

- Blumberg LJ, Humphries JE, Jones SD, Pearce LB, Holgate R, Hearn A, et al. Blocking FcRn in humans reduces circulating IgG levels and inhibits IgG immune complex-mediated immune responses. *Sci Adv* 2019;5:eaax9586.
- Howard JF Jr, Bril V, Burns TM, Mantegazza R, Bilinska M, Szczudlik A, et al. Randomized phase 2 study of FcRn antagonist efgartigimod in generalized myasthenia gravis. *Neurology* 2019;92:e2661–73.
- Huang YH, Kuo CF, Chen YH, Yang YW. Incidence, mortality, and causes of death of patients with pemphigus in Taiwan: a nationwide population-based study. *J Invest Dermatol* 2012;132:92–7.
- Kasperkiewicz M, Ellebrecht CT, Takahashi H, Yamagami J, Zillikens D, Payne AS, et al.

- Pemphigus. *Nat Rev Dis Primers* 2017;3:17026.
- Langan SM, Smeeth L, Hubbard R, Fleming KM, Smith CJ, West J. Bullous pemphigoid and pemphigus vulgaris—incidence and mortality in the UK: population based cohort study. *BMJ* 2008;337:a180.
- Newland AC, Sánchez-González B, Rejtő L, Egyed M, Romanyuk N, Godar M, et al. Phase 2 study of efgartigimod, a novel FcRn antagonist, in adult patients with primary immune thrombocytopenia. *Am J Hematol* 2020;95:178–87.
- Patel DD, Bussell JB. Neonatal Fc receptor in human immunity: function and role in therapeutic intervention. *J Allergy Clin Immunol* 2020;146:467–78.
- Qiao SW, Kobayashi K, Johansen FE, Sollid LM, Andersen JT, Milford E, et al. Dependence of antibody-mediated presentation of antigen on FcRn. *Proc Natl Acad Sci USA* 2008;105:9337–42.
- Tacke CE, Smits GP, van der Klis FR, Kuipers IM, Zaaijer HL, Kuijpers TW. Reduced serologic response to mumps, measles, and rubella vaccination in patients treated with intravenous immunoglobulin for Kawasaki disease. *J Allergy Clin Immunol* 2013;131:1701–3.
- Werth VP, Culton DA, Concha JSS, Graydon JS, Blumberg LJ, Okawa J, et al. Safety, tolerability, and activity of ALXN1830 targeting the neonatal Fc receptor in chronic pemphigus. *J Invest Dermatol* 2021;141:2858-65.e4.

The role of LL-37 in cutaneous immune regulation

The pleiotropic role of LL-37 in the cutaneous response to pathogen-associated molecular patterns (PAMPs) is rapidly emerging. LL-37 is the C-terminal domain of the sole human cathelicidin precursor, hCAP18, which is cleaved by kallikrein 5 in response to UV, tissue injury, and microbial infection (Moreno-Angarita et al., 2020). The effector cathelicidin, LL-37, has been shown to directly impair microbial function as a pore-forming toxin (Xhindoli et al., 2016). In addition to its bactericidal activity, LL-37 both inhibits IL-1B and amplifies proinflammatory signaling through lipopolysaccharide (LPS) binding in vitro and in vivo (Dombrowski et al., 2011; Hu et al., 2014). LL-37 has also been shown to be upregulated in the skin of patients with psoriasis, suggesting an overarching relevant role in the mediation of cutaneous inflammatory disease (Lande et al., 2007).

LL-37 promotes inflammasome activation with LPS costimulation

On the basis of the previous conflicting reports of LL-37 action on IL-1 signaling and inhibition as well as the synergistic amplification of LPS and other PAMPs, Yoon et al., 2021 treated bone marrow-derived macrophages with LL-37 in the presence and absence of LPS. They found that LPS priming followed by treatment of LL-37 induced caspase-1 cleavage and IL-1B production, whereas treatment with LL-37 and LPS alone as well as with LL-37 primed by TNF- α and IFN- γ was insufficient in activating inflammasomes. This was also seen in dendritic cells (DCs) and

See related article on pg 2885

Cathelicidin LL-37 Ignites Primed NLRP3 Inflammasomes in Rosacea

David O. Croitoru¹ and Vincent Piguet^{1,2}

Microbes and commensal mites contribute to the development of inflammation and neurovascular dysregulation in rosacea. Cathelicidin family proteins are epithelial antimicrobial peptides expressed in higher-order mammals. In humans, mature LL-37 is cleaved from its precursor in response to microbial infection, UV light, and injury. In their new article in the *Journal of Investigative Dermatology*, Yoon et al. expand on existing evidence supporting LL-37 proinflammatory activity in lipopolysaccharide (LPS)- and UV-primed models of rosacea. They show in vitro that LL-37 promotes NLRP3-mediated inflammasome activation through lysosomal destabilization in the presence of LPS and that the injection of LL-37 in vivo leads to skin inflammation that is abrogated by direct NLRP3 inhibition and homozygous knockout in a murine model.

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“Repeated exposure to LL-37 can activate the NLRP3-mediated inflammasome pathway, which in turn promotes the recruitment of inflammatory cells and skin inflammation, triggering rosacea-like phenotypes.”

Clinical Implications

Rosacea is a chronic, inflammatory disorder typically involving centrofacial skin, which may have a profound effect on QOL through pruritus, pain, cosmeseis, and potentially, permanent disfigurement in certain clinical variants (Elsner et al., 2004). It is a common skin disease, with an estimated prevalence of 5.5% among the global adult population. There are mixed reports of female preponderance, with typical onset after the age of 30 years (Gether et al., 2018). Commensal microbes (*Staphylococcus epidermidis*) and mites (*Demodex follicularum*) have been implicated in the pathogenesis from both translational and clinical standpoints, supported by the efficacy of antimicrobials (tetracycline, nitroimidazole) and antimitotic therapies (ivermectin) (Lazaridou et al., 2011). Despite this, the mechanisms of action of exogenous triggers and the associated host inflammatory mediators remain unknown, and no targeted host-directed therapies yet exist.

bone marrow-derived neutrophils, suggesting a bona fide role for LL-37 in immune cell inflammasome activation through toll-like receptor (TLR) 3 and/or TLR4 priming. Furthermore, they show that cytosolic entry of LL-37 may be mediated by P2X7 receptor-mediated endocytosis, which is disrupted by oxidized adenosine triphosphate.

Intradermal LL-37 induces NLRP3-dependent inflammasome activation and skin inflammation

Cleavage of caspase 1 and IL-1 β was detected in lesional skin of wild-type (WT) mice (C57BL/6) but not in that of *NLRP3*^{-/-} mice after intradermal injection of LL-37, and it was absent in nonlesional control skin in both strains. Phenotypically, this correlated with rosacea-like inflammation (modified skin thickness and erythema) and lesional accumulation of Gr1+ macrophages and DCs in WT mice compared with that in *NLRP3*^{-/-} mice. There was limited inflammation in both *NLRP3*^{-/-} and WT mice with phosphate-buffered intradermal controls. Further supporting the necessary role of NLRP3 in vivo, ASC protein oligomerization was detected in the same pattern after injection, and this was also replicated by a caspase-1 cleavage-activated fluorophore.

Lysosomal rupture and potassium leakage may underly LL-37 NLRP3 activation

Potassium efflux is a unique feature of NLRP3 inflammasome activation, and transmembrane concentration gradients were found to be significantly

associated with inflammasome activation, attenuated by glibenclamide and high extracellular potassium hydrochloride. Lysosomal rupture was also detected through lysosomal protease cathepsin B, which was additionally associated with inflammasome activation.

Inhibition and knockout of NLRP3 attenuates the murine LL-37 rosacea phenotype

Selective inhibition of NLRP3 with MCC950, a small molecule inhibitor, showed a pronounced decrease in the LL-37 murine rosacea-like phenotype and marked decrease in IL-1B, with normal endocytosis of LL-37 in vitro. Taken together, the inflammasome-mediated effects of LL-37 appear to be initiated by TLR3 and TLR4 priming and downstream of NLRP3 inflammasome activation, which may be mediated by impaired lysosomal trafficking and cytosolic rupture.

LL-37 as an inflammatory network regulator with multiple effectors

The detection of LL-37 in high concentrations in the skin of patients with rosacea compared with that of healthy controls (Yamasaki et al., 2007), its implication in other inflammatory diseases of the skin, and the preserved immune function of homologous cathelicidin family proteins across species support LL-37 as a potential link between exogenous PAMPs and host autoinflammation. This work by Yoon et al., 2021 aims to reconcile the previously reported inhibitory and synergistic roles of LL-37 in LPS-induced IL-1B

and caspase cleavage. They show that only in the setting of LPS-primed TLRs does LL-37 achieve significant cytosolic access through P2X7 receptors and activate the inflammasome through NLRP3 in a mechanism that implicates lysosomal rupture and potassium efflux. Further validation of this work using gram-positive and gram-negative microbes will provide additional insight into the physiologic relevance of LPS in the context of LL-37 and, subsequently, into the taxa of bacteria most responsible for LL-37-driven rosacea inflammation. The observation that LL-37 alone was sufficient to induce inflammasome activation and a rosacea-like phenotype in vivo but not in vitro suggests that other signals occurring in the murine cutaneous microenvironment may fulfill the role of LPS TLR priming. This is another attractive area for future investigation. The limitations of this model must also be recognized, and ultimately, the physiologic role of LL-37 in rosacea, while potentially still mediated by NLRP3, may be shifted toward different driving factors and mediators in humans. The identification of NLRP3 and the inflammasome as therapeutic host targets may have immediately translatable effects in the treatment of rosacea, generally, or may be of particular relevance to certain clinical variants (i.e., granulomatous). Future animal and human models will be critical to exploring the relevance of this interaction and the utility of the pathway as a clinical target.

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CONFLICT OF INTEREST

VP has no personal financial ties with any pharmaceutical company. He undertakes advisory work for Pfizer, AbbVie, Janssen, UCB, Novartis, Almirall, and Celgene. He has received honoraria from Kyowa Kirin. In his role as Department Director of Dermatology at the University of Toronto (Ontario, Canada), VP has received departmental support from AbbVie, Bausch Health, Celgene, Janssen, LEO Pharma, Lilly, NAOS, Novartis, Pfizer, Pierre-Fabre, and Sanofi in the past 36 months. DOC states no conflict of interest.

REFERENCES

Dombrowski Y, Peric M, Koglin S, Kammerbauer C, Göss C, Anz D, et al. Cytosolic DNA triggers inflammasome activation in

- keratinocytes in psoriatic lesions. *Sci Transl Med* 2011;3:82ra38.
- Ellsner A, Duncan M, Gavrilin M, Wewers MD. A novel P2X7 receptor activator, the human cathelicidin-derived peptide LL37, induces IL-1 beta processing and release. *J Immunol* 2004;172:4987–94.
- Gether L, Overgaard LK, Egeberg A, Thyssen JP. Incidence and prevalence of rosacea: a systematic review and meta-analysis. *Br J Dermatol* 2018;179:282–9.
- Hu Z, Murakami T, Suzuki K, Tamura H, Kuwahara-Arai K, Iba T, et al. Antimicrobial cathelicidin peptide LL-37 inhibits the LPS/ATP-induced pyroptosis of macrophages by dual mechanism. *PLoS One* 2014;9:e85765.
- Lande R, Gregorio J, Facchinetti V, Chatterjee B, Wang YH, Homey B, et al. Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. *Nature* 2007;449:564–9.
- Lazaridou E, Giannopoulou C, Fotiadou C, Vakirlis E, Trigoni A, Ioannides D. The potential role of microorganisms in the development of rosacea. *J Dtsch Dermatol Ges* 2011;9:21–5.
- Moreno-Angarita A, Aragón CC, Tobón GJ. Cathelicidin LL-37: a new important molecule in the pathophysiology of systemic lupus erythematosus. *J Transl Autoimmun* 2020;3:100029.
- Xhindoli D, Pacor S, Benincasa M, Scocchi M, Gennaro R, Tossi A. The human cathelicidin LL-37 — a pore-forming antibacterial peptide and host-cell modulator. *Biochim Biophys Acta* 2016;1858:546–66.
- Yamasaki K, Di Nardo A, Bardan A, Murakami M, Ohtake T, Coda A, et al. Increased serine protease activity and cathelicidin promotes skin inflammation in rosacea. *Nat Med* 2007;13:975–80.
- Yoon S-H, Hwang I, Lee E, Cho H-J, Ryu JH, Kim T-G, et al. Antimicrobial peptide LL-37 drives rosacea-like skin inflammation in an NLRP3-dependent manner. *J Invest Dermatol* 2021;141:2885–94.e5.

“Pharmacological inhibition of either mitochondrial fission or mitophagy effectively restored the sensitivity of melanoma cells to ER stress induction.”

control because UPR induction rewires mitochondrial fission and fusion dynamics and initiates the clearance of dysfunctional mitochondria through mitophagy (Senft and Ronai, 2015). Continual cell division coupled with a tumor microenvironment containing limited nutrient and oxygen perfusion strains protein folding within cancer cells. The resulting high levels of ER stress in tumor cells distinguish them from most untransformed cells and provide a potential opportunity for a therapy capable of discriminating tumor selectivity (Ron and Walter, 2007). Whereas aggravating ER stress or inhibiting the UPR has been promising in some tumor types, melanoma cells appear to be relatively resistant. In their new article, Wang et al. (2021) investigate whether a particular cotargetable pathway could sensitize melanoma cells to ER stress-induced apoptosis (Figure 1).

Wang et al. take a comparative approach, identifying a panel of cell lines that have different sensitivities to ER stress induction by both tunicamycin and thapsigargin treatment. Although chemical treatment of all profiled lines induced UPR, cell death responses stratified sensitive and resistant lines. Transcriptional analysis revealed that the genes encoding UPR-signaling proteins were anticorrelated with mitochondrial genes in melanoma, and staining of human melanoma tumors corroborated this. The authors hypothesized that mitochondrial health might buffer cells from ER stress and found that chemical ablation of mitochondrial ROS rescued sensitive cells from cell death on ER stress induction.

An analysis of mitochondrial morphology further implicated mitochondrial fission and fusion dynamics in determining sensitivity to ER stress induction. Cells that were more resistant

See related article on pg 2932

MFN2 Stabilization: A Bridge for Endoplasmic Reticulum Stress Sensitivity in Melanoma



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In a new article in the *Journal of Investigative Dermatology*, Wang et al. (2021) report that mitochondrial quality control modulates responses to endoplasmic reticulum (ER) stress in melanoma. They implicate a linear pathway of XBP1, MARCH5, and MFN2 that act together to regulate mitochondrial fission and mitophagy and ultimately mediate melanoma cell sensitivity to ER stress. This work informs therapeutic combinations and biomarker strategies for targeting melanoma organellar homeostasis as well as for life–death decisions.

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Protein folding quality control and the initiation of glycosylation occur in the endoplasmic reticulum (ER) shortly after secreted and integral membrane proteins are translated into its lumen (Ron and Walter, 2007). Three parallel signaling apparatuses monitor protein processing in the ER and initiate a series of signaling events known as the unfolded protein response (UPR) on the

disruption of ER proteostasis (Ron and Walter, 2007). In most contexts, UPR-driven transcriptional programs are prosurvival and include factors that slow the rate of protein synthesis and upregulate molecular chaperones (Hill et al., 2014; Ron and Walter, 2007). Critical to the findings of Wang et al. (2021), UPR activation also feeds into mitochondrial health and quality

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