Reviewer's report

Title: Toll-Like receptor 2 activation and comedogenesis: implications for the pathogenesis of acne

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Reviewer: Bodo Melnik

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Review of BMC Dermatology manuscript
(research article)

Toll-like receptor 2 activation and comedogenesis: Implications for the pathogenesis of acne
Joanne Louise Selway, Tomasz Kurczab, Terence Kealy and Kenneth Langlands

This paper studies the role of ligand activated Toll-like receptor 2 (TLR2) and TLR4 in interleukin-1alpha (IL-1a)-mediated comedo formation. The authors re-examine the concept of Ingham et al. (1992) Pro-inflammatory levels of interleukin 1alpha-like bioactivity are present in the majority of open comedones in acne vulgaris. J Invest Dermatol 98:895-901, who suggested two decades ago that IL-1-like activity may play a role in comedogenesis.

The key messages of this paper is that PAMP-activated TLRs may induce hypercornification in sebaceous follicles maintained ex vivo. However, the authors only provide indirect evidence that increased expression of IL-1a may play a role in comedogenesis.

There are several weaknesses in Methods, Results and Discussion, which have to be addressed.

Methods
The authors should describe the exact procedure of IL-1a treatment of sebaceous glands ex vivo in this section.

Experimental Design
The ligand stimulation of TLRs presumably results in comedo formation, which is thought to be driven by TLR-stimulated IL1-a expression. Although TLR blocking by antibodies prevented cornification, a direct negative control using a IL-1a blocking antibody has not been presented.

Results
The authors do not present statistically significant morphometric data of the number of sebaceous glands with comedo-like cornification. The reviewer would like to know how many sebaceous glands showed a transformation into comedo
like structures. A classification of the keratin types would be most interesting. Only one picture is presented in this study, which shows a comedo-like stricture. This is not convincing to the reviewer. Do all histologically controlled ex vivo sebaceous glands produce these comedo-like structures?

Discussion

A further concern of ex vivo sebocyte organ cultures is the fact that they are removed from the normal skin environment and immune system including TLR expressing monocyte-macrophages. This disadvantage of explant sebocyte cultures should be discussed.

The P. acnes-PAMP-TLR2-IL-1a pathway of comedogenesis or even acne pathogenesis appears to be oversimplified. IL-1a, is a ubiquitous interleukin expressed by various inflammatory skin diseases. IL-1a is upregulated by skin barrier perturbations or skin occlusion, conditions which are not associated with the development of comedones. For instance, follicular hypercolonization of pityrosporum ovale or gram-negative folliculitis, which obviously induce TLR stimulation do not promote comedogenesis. There are no comedones in gram-negative folliculitis.

The idea that IL-1a induces sebocyte involution and depression of sebum production, thereby suppressing P. acnes growth is disputable. TLR signaling promotes IL-1 and TNFa expression and in addition activates cell proliferation via increased PI3K/Akt/mTORC1 signalling (see the excellent recent review of Li X et al. (2010) Toll-like receptor signaling in cell proliferation and survival. Cytokine 49:1-9). Furthermore, Choi et al. provided recent evidence that TNFa, promoted sebocyte growth and stimulated sebaceous lipogenesis (Choi JJ et al. TNF-# increases lipogenesis via JNK and PI3K/Akt pathways in SZ95 human sebocytes. J Dermatol Sci 2012: 65: 179-188).

Thus, the reviewer suggests that the sebocyte organ culture experiments are extended and should include data on TNFa expression to receive a more realistic picture of the scenario.

The reviewer proposes the following sequelae of events:

Increased IGF-1 signaling of puberty superimposed by insulinotrophic Western diet (carbohydrates and milk consumption) results in sebaceous gland hyperplasia and increased sebum production (Melnik B (2012) Dietary intervention in acne: Attenuation of increased mTORC1 signaling promoted by Western diet. Dermatoendocrinol. 4(1):20-32).

Increased growth promoting mTORC1 signaling of Western diet may not only induce sebaceous lipid biosynthesis but may also affect FoxO-regulated innate immunity. High insulin/IGF-1 signaling is known to suppress the activity of FoxO transcription factors, which control innate immunity and the expression of antimicrobial peptides independent of PAMP-triggered TLR signaling (Becker T et al. (2010) FOXO-dependent regulation of innate immune homeostasis. Nature 463(7279):369-373).
A decrease of FoxO-mediated innate immunity may allow the overgrowth of P. acnes, which than after reaching a critical “threshold” for activation of TLR-driven immune responses, may activate the proinflammatory and pro-proliferative signal transduction of sebocytes, keratinocytes as well as infiltrating monocyte macrophages.

Increased availability of sebum (sebum fatty acids) promotes the growth and probably biofilm formation of P. acnes (Jahns et al. (2012) An increased incidence of Propionibacterium acnes biofilms in acne vulgaris: a case-control study. Br J Dermatol 167(1):50-58). It is well known that bacteria growing as biofilms are of higher virulence and thus may more profoundly increase P. acnes-PAMPs-mediated activation of TLR of sebaceous glands, infiltrating macrophages and follicular keratinocytes resulting in increased expression of IL-1α, TNFα and other pro-inflammatory cytokines observed in acne lesions.

TLR-mediated increase in IL-1α could of course amplify inflammation and expression of TNFα, which by activation of the IKKbeta-TSC1 pathway may further increase sebaceous hyperplasia and sebum production (Lee DF et al. (2007) IKK beta suppression of TSC1 links inflammation and tumor angiogenesis via the mTOR pathway. Cell130: 440-455; Dan HC et al. (2008) Akt-dependent regulation of NF-{kappa}B is controlled by mTOR and Raptor in association with IKK. Genes Dev 22: 1490-1500

It should be kept in mind that benzoyl peroxide treatment of acne significantly reduces P. acnes numbers within seconds or minutes. TLRs pathways respond quickly and should thus be downregulated at least within several days after P. acnes extinction. However, clinical treatment with BPO needs weeks to induce clinical improvements of acne. Thus, not BPO-decreased P. acnes-TLR-signaling plays a central role in acne pathogenesis but more likely ROS-mediated increases in FoxO-regulation and FoxO-mediated control of innate immunity (Melnik BC, Zouboulis CC (2013) Potential role of FoxO1 and mTORC1 in the pathogenesis of Western diet-induced acne. Exp Dermatol DOI: 10.1111/exd.12142).

Incomplete list of references

The authors should consider the paper of Lebre MC et al. (2007) Human keratinocytes express functional Toll-like receptor 3, 4, 5, and 9. J Invest Dermatol 127:331-341 and should compare theses data with their own data on TLR expression on keratinocytes.

The reviewer appreciates if the authors could integrate these lines of thought and would refrain from considering comedogenesis as a simple P. acnes-induced TLR-IL-1α-driven process. In fact, nature appears to be more complicated.

Best regards,
Bodo C. Melnik

Level of interest: An article of importance in its field
Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
I declare that I have no competing interests