Innate immunity and Toll-like Receptors modulation in acne

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**Summary**

**BACKGROUND.** Toll-like receptors (TLR) are trans-membrane receptors part of the innate immune system. Defects in TLR pathway have the potential to lead to increased susceptibility to dysregulation and play a role in the pathogenesis of numerous inflammatory skin diseases (as seborrheic dermatitis, acne, atopic dermatitis). Toll-like receptor 2 (TLR2) can be activated by peptidoglycans from P. acnes structure, with a subsequent inflammatory cascade involving pro-inflammatory interleukins (IL-8) and inducing human beta-defensein 2 (hBD2) as one of the innate immune mechanisms of defense against microbes.

**PURPOSE.** We sought to assess the effects of a vegetal natural extract (Ombelliferae) associated with a long chain lipid (TLR2-Regul®) in *ex vivo* human skin in contact with *P. acnes*, focusing on the markers of TLR2 activation: expressions of IL-8 and hBD2. In a second step we assessed the effects of the same emulsion in a double blind clinical trial in a series of patients with light inflammatory acne.

**METHODS.** Normal human skin explants were treated by an emulsion O/W formulated with the complex TLR2-RegulTM (20 µl per explant) or by its vehicle (control). Extracts of *P. acnes* were added. The dosages of IL-8 (ELISA) and of hBD2 were performed at 24h. In a second part of this study was made a double blind clinical trial including acne patients with light inflammatory acne (2-3 IGA scale) using same O/W emulsion formulated with TLR2-Regul® (compared to its vehicle): 2 applications/day during 12 weeks. Were excluded patients having systemic or topical treatment for acne.

**RESULTS.** *P. acnes* induced significant increase of IL-8 expression in all untreated skin explants. Skin explants previously treated by TLR2-Regul® in contact with *P. acnes* had a significant less important expression of IL-8 compared to control (p<0.01) and a significant increase of hBD2 expression (p<0.01).

**CONCLUSIONS.** In normal human skin explants in contact with microbial extracts, the expression of IL-8 (as main cytokine linked to TLR2 activation) was increased. In all skin explants treated by the complex TLR2-Regul® and exposed to extract of *P. acnes*, the expression of IL-8 was significantly down-regulated and hBD2 expression was significantly up-regulated. Results of the clinical trial showed in a series of 34 acne patients a significant decrease of IGA mean score (by 40%) in the group verum compared to the vehicle group (-24%)(p<0.05). Larger clinical trials are ongoing in order to confirm the results of this pilot study focusing on the modulation of TLR2 in acne patients.

**Key words:** Toll-like receptor 2, IL-8, betadefensin hBD2, inflammatory acne.

**Introduction**

The particular field that was subject of the highest award in medical research in 2011 underlined once again the importance of innate immunity and of Toll-Like Receptors (TLRs). This domain of medical research was opened more than 15 years ago by Nobel Prize recipients *Jules Hoffman* who discovered TLRs in *Drosophila* flies¹ and *Bruce Beutler* that focused on the role of TRLs in mammals².

With the discovery of TLRs, the understanding of the innate immunity role was widened from the previously described “nonspecific” mechanisms - as the activation of complement and the phagocytosis - to the complex pathways of the TLRs activation and the subsequent expression of pro-inflammatory molecules and of anti-microbial peptides, subject of several recent studies.

This article presents in the first part *ex vivo* studies on TLR2 activation by *P. acnes* in human skin and its topical regulation by an association of a vegetal extract (*Ombelliferae*) with a long-chain lipid (TLR2-Régul®), then in the second part the results of a double blind, pilot clinical trial, using same topical formulation in a series of acne patients.
Toll-Like Receptors

The innate immune system is activated by complex mechanisms, among them TLRs are trans-membrane cellular receptors of human main “interface” tissues as skin, gastrointestinal tract and lungs. Skin TLRs are expressed by keratinocytes, Langerhans’ cells, macrophages, monocytes and granulocytes. TLRs are activated by their contact with ligands from pathogen-associated molecular patterns (PAMPs) of microbes (bacteria, yeasts or viruses). Up today more than 11 types of TLRs were described 3,4 (Figure 1).

TLRs have an extracellular domain (leucine) that is able to recognize ligands from microbes’ structures, and an intracellular domain of the receptor for IL-1 and protein MyD88 Toll-IL-1R homology (TIR) from cytoplasm (Figure 2). The TIR domain participates to the induction of the cellular response (pro-inflammatory cytokines and antimicrobial peptides up-regulation). The binding process between microbe structures and TLRs’ extracellular domain triggers a signal that initiates a succession of steps involving specific inflammation messengers, resulting in the release of various cytokines and defensines. Toll-like receptor 2 (TLR-2) is activated by 3 compounds of microbes as *P. acnes*. This will trig an inflammatory cascade involving mainly IL-8 (but also other cytokines as IL-1, IL-6) 3.6.

Inflammatory response triggered by microbes as *P. acnes, S. aureus, Malassezias*, human papilloma viruses - HPV is linked to the activation of TLRs – especially in inflammatory diseases with microbial aggravating factors as acne, seborrheic dermatitis, atopic dermatitis 3.5.

*P. acnes*-induced inflammation in acne is complex, a part of inflammatory pathway is due to TLR2.
activation. Same receptor is activated in atopic dermatitis by *S. aureus* in leprosy borreliosis / Lyme disease (ligands from *Borrelia burgdorferi*) Yeasts-induced inflammation can be found in seborrhoeic dermatitis (linked to *Malassezia*’ zymosan activating TLR2) and candidiasis (*Candida species* activate same TLR). *Papilloma viruses* from warts activate TLR7, and TLR8.

In psoriasis TLR2, TRL7, TLR8, TLR9 are activated. In acne TLR participate in innate response to microbial presence: wall structure of *P. acnes* (mainly glyco-peptides) are acting as ligands and activate TLR2 (and TLR4). *P. acnes* activates TLR-2 and consequently induces the expression of IL-8 and IL-6 within follicular keratinoctyes, and of IL-8 and IL-12 within macrophages. Sebocytes’ and keratinocytes’ antimicrobial peptides expression can be up-regulated, this being an important contribution to the innate immunity response within the pilo-sebaceous follicle. Changes of sebum lipids - as a modified ratio between different lipid fractions - induce alterations of keratinocyte in terms of differentiation and IL-1 secretion, leading to infra-infundibular hyperkeratosis and initiating first steps of micro-comedo induction. It has been shown that keratinocytes can respond to bacterial, fungal, and viral pathogens that have breached the stratum corneum by producing 2 important classes of endogenous peptides, beta-defensins and cathelicidins. Beta-defensin 1 is constitutively expressed, beta-defensin 2 (hBD2) is strongly up-regulated upon antigenic stimulation. In acne other factors can also increase hBD2 expression as free fatty acids from sebum.

**TLR modulation in human skin**

We previously showed that in human skin in contact with microbial extracts the expression of IL-8 significantly linked with the activation of TLR2. We present here ex vivo tests on human skin assessing the modulation of TLR2 in presence of *P. acnes* with the focus on IL-8 and also on antimicrobial peptide expression hBD2.

An association of a natural vegetal extract (from the family of *Ombelliferae*) with a synthetic long-chain lipid (TLR2-Regul®) was tested on ex vivo human normal skin in contact with purified microbial extract of *P. acnes*.

The objective of this study was to assess the effects of the TLR2-Regul® on the expression of IL-8 – considering that this interleukin is not specific for TLR2 activation but is one of the main cytokines expressed after the activation of TLR2 in keratinocytes in contact with microbial extracts. This first test was followed by the assessment of antimicrobial peptide hBD2 expression in human skin in contact with *P. acnes* in the presence of same complex TLR2-Regul®.

**Methods:** skin explants A. were incubated (1 h at 37°C) in absence (control) or in presence of an emulsion O/W formulated with the complex TLR2-Regul® (20 µl per explant); B. at 1 h were added inactivated extracts of *P. acnes* (20 µl per explant); C. after 24 h incubation was performed the dosage of IL-8 (ELISA). Same steps A and B were used in order to assess antimicrobial peptides expression in human skin explants, in last step C. after 24 h incubation was performed the dosage of human beta-defensin 2 (hBD2) (ELISA). Comparisons for each type of test were made between skin explants coming from same donor. A difference of IL-8 or hBD2 expression less that 0.01 was considered as significant.

**Results:** microbial extract of *P. acnes* induced a significant increase of IL-8 expression in all untreated skin explants. Skin explants treated by TLR2-Regul® in contact with microbial extracts had a significant less important expression of IL-8 compared to control (p<0.01) (Figure 3). Skin explants in contact with *P. acnes* pre-treated with TLR2-Regul® had a significant up-regulated expression of hBD2 compared to untreated explants in contact with the same microbial extract (p<0.01) (Figure 4).

In these studies on human skin explants previously treated by the TLR2-Regul® and exposed to *P. acnes* extract, the expression of IL-8 was significantly decreased compared to untreated skin (p<0.01), antimicrobial peptide hBD2 expression was significantly up-regulated in skin treated with TLR2-Regul® in contact with *P. acnes* compared to untreated skin.
Clinical application of TLR2 modulation in inflammatory acne

The purpose of this in vivo pilot study was to assess the effects of TLR2-Regul® in a double blind clinical trial study in patients with light inflammatory acne.

Methods: TLR2-Regul® was included in a formula of an O/W emulsion (at 1%) and tested in monotherapy in acne patients with light inflammatory forms. Were included adult acne patients (above 18 years old) with light inflammatory acne on IGA scale, rated 2 to 3 on the 5-point investigator global assessment of acne severity scale.

Included patients were randomized and treated with verum or by its excipient. Were excluded patients with IGA scores less than 2 or over 3, patients under topical and/or systemic treatment for acne or having stopped their treatment less than 4 weeks before baseline. Daily cleansers used by the patients during the trial were “neutral” (no anti-acne effect).

Results: were included 34 adult acne patients, 16 in the group A (verum) and 18 in group b (excipient), with a mean score of acne IGA score of 2.3 in group A vs 2.2 in group B.

Inflammatory lesions decreased significantly at 3 months in the group verum (mean IGA decreased by 40% compared to baseline) compared to its excipient (mean IGA decreased by 24% compared to baseline) (p<0.05) (Figure 5). Skin tolerance was very good in both groups.
Conclusions

Human skin explants in contact with *P. acnes* express increased levels of IL-8, in skin explants pre-treated with TLR2-Regul® emulsion the expression of IL-8 was less important compared to control (p<0.01). In pre-treated skin with TLR2-Regul® in contact with *P. acnes* the expression of anti-microbial peptide hBD2 was up-regulated. In this double blind, pilot clinical trial in a series of 34 patients with light inflammatory acne (IGA score 2 to 3), the group treated with the emulsion based on TLR2-Regul® showed at week 12 a significant decrease of inflammatory lesions compared to excipient group (p<0.05). As a recently developed area of research, the interest for the innate immunity is continuously growing in dermatology, especially with the opening of new and therapeutic prospective targeting TLRs. The development of active ingredients that are able to modulate the innate response can open new ways of treatment in the field of inflammatory skin diseases aggravated by microorganisms as acne. Larger clinical trials are ongoing in order to confirm the results of the pilot trial presented in this article. Most recently, the topical calcineurin antagonists such as tacrolimus 16 and pimecrolimus 17, 18 have been successfully used. Thus, they should be considered as alternative or adjuvant therapies for patients who do not respond to traditional treatment. Otherwise, prevention becomes a priority in the management of SIRD. It may be a recalcitrant problem, imposing a great psychological stress on the patients.

Disclosures:

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References


