IL-36γ Is Involved in Psoriasis and Allergic Contact Dermatitis

References


Abbreviations: ACD, allergic contact dermatitis; KC, keratinocyte; NiSO4, nickel sulfate; PBMC, peripheral blood mononuclear cell; Ra, Receptor antagonist; IL-36Ra, recombinant interleukin-36 receptor antagonist; TNF, tumor necrosis factor

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Supplementary Material

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at http://dx.doi.org/10.1016/j.jid.2016.03.001.

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TO THE EDITOR

We read with great interest the article by D’Erme et al. (2015) entitled “IL-36γ (IL-1F9) Is a Biomarker for Psoriasis Skin Lesions.” By performing an unsupervised gene cluster analysis, the authors identified genes specifically expressed within different subsets of inflammatory skin diseases; among these, psoriasis-associated genes were further analyzed to show that IL-36γ showed the strongest association with psoriasis when compared with the other skin diseases. The authors confirmed this specificity, showing high levels of IL-36γ both in skin by immunohistochemistry and in serum by ELISA assay. A good correlation was found between this marker (based on serum levels) and the severity of the disease, with a decline after effective anti-tumor necrosis factor (TNF)-α treatment. Moreover, they reported that IL-36γ has a highly specific positive predictive diagnostic value for psoriasis. Hence, the results presented in the article propose IL-36γ as a valuable biomarker in psoriatic patients, both for diagnostic purposes and for measurement of disease activity.

The findings of D’Erme et al. (2015) are very intriguing, and we strongly believe that IL-36α, IL-36β, and IL-36γ are involved in the pathogenesis of psoriasis but also in allergic contact dermatitis (ACD). As we have previously shown, IL-36α, IL-36β, and IL-36γ were enhanced in psoriasis as well as in ACD skin (Balato et al., 2013; Mattii et al., 2013). We speculate that they might represent markers of skin inflammation in general, and it seems weakly reliable that IL-36γ alone might constitute a specific psoriasis index. However, to support our thesis we performed further investigations. The experimental protocol was approved by the ethics committee of our institution and conformed to the principles outlined in the Declaration of Helsinki. Each subject gave written informed consent before entering the study. Characteristics of allergic contact dermatitis (ACD) recruited patients are showed in Supplementary Table S1 online. First, we compared skin IL-36 gene expression in subjects with psoriasis or ACD and in healthy control subjects and found a significant increase for all subjects in both disease groups compared with healthy subjects, except for IL-36 receptor antagonist (IL-36Ra) in ACD (Figure 1a). IL-36Ra is the anti-inflammatory member of IL-36 subfamily, and the lack of the natural antagonist in ACD led us to hypothesize that the activity of IL-36α, IL-36β, and IL-36γ is unopposed and therefore that these cytokines can drive skin inflammation (Mattii et al., 2013). Nevertheless, we found that IL-36γ was expressed at a higher level in psoriatic skin versus ACD skin (Figure 1a). This finding was in agreement with Quaranta et al. (2014), who found a significant increase of IL-36γ in both psoriatic and eczematous skin, with a main up-regulation in psoriasis. The effects of an anti-TNF-α treatment on psoriatic skin levels of IL-36 (α, β, and γ) and IL-36Ra were evaluated, and we found a significant decrease for all analyzed interleukins except for IL-36β. These data reflect the fact that an efficacious...
treatment is able to down-regulate all the pro- and anti-inflammatory members of IL-36 subfamily indiscriminately (Figure 1b). Next, we analyzed IL-36γ serum levels in the same two categories of patients and found no significant differences, even when comparing with healthy control subjects (Figure 1c); this result was confirmed by the presence of a positive control checked by Western blot analysis as well (see Supplementary Figure S1 online). The positive control was supernatant of healthy skin treated with TNF-α. Obviously, we didn’t find any correlation with psoriasis severity (data not shown). Because our findings are in contrast with the results of D’Erme et al. (2015), particularly regarding IL-36γ serum levels, we deeply investigated this point. First, we analyzed IL-36γ gene expression in circulating peripheral blood mononuclear cells (PBMCs) isolated from psoriatic and ACD patients as well as healthy control subjects and found no significant differences (Figure 1d). Moreover, to further elicit a response, PBMCs isolated from healthy subjects and from psoriatic and nickel-ACD patients were exposed to concanavalin A, toxic shock syndrome toxin, and nickel sulfate (NiSO₄). Only the stimulation with NiSO₄ in ACD PBMCs was able to induce a significant increase of IL-36γ with respect to healthy control subjects (Figure 2a), suggesting that this IL might be involved in ACD, too. Afterward, IL-22, an IL known to be involved in both psoriasis and ACD (Hao, 2014; Cavani et al., 2012), was analyzed in the same experimental setting, with the addition of recombinant IL-36Ra (rIL-36Ra) treatment to analyze whether IL-22 expression could be hampered by blocking IL-36 action. IL-22 was enhanced after stimulation with toxic shock syndrome toxin and moderately down-regulated by rIL-36Ra in healthy subjects (not significant) and in subjects with psoriatic and nickel-ACD PBMCs (Figure 2b), whereas no increase was observed after NiSO₄ treatment in all three categories of subjects with subsequently no effects by rIL-36Ra (Figure 2b). At this point, we wanted to highlight IL-36γ involvement in ACD, and ex vivo ACD non lesional skin assay was performed. IL-36γ expression was significantly augmented in uninvolved ACD skin when treated with the allergen (NiSO₄), but an even more enhanced increase was obtained in uninvolved ACD skin when PBMCs were added to the system (Figure 2c). To comprehend the contribution of PBMCs, we compared IL-36γ gene expression and that of its known inducers (TNF-α and IL-1α) in nickel-ACD PBMCs treated in vitro with NiSO₄ and in nickel-ACD PBMCs incubated in ex vivo ACD nonlesional skin assay. A significant increase was detected for all ILs in PBMCs that had been incubated in the skin system (Figure 2d).

It is well known that skin inflammation results from the multipartite interactions of, at least, keratinocytes (KCs), T cells, and antigen-presenting cells. In this scenario, KCs are the major source of IL-36γ, which contributes to skin inflammation by acting on KCs and antigen-presenting cells and indirectly upon T cells driving tissue

Figure 1. IL-36 expression in psoriasis and allergic contact dermatitis. (a) IL-36 (α, β, and γ) and IL-36Ra (Ra) gene expression in lesional psoriatic skin (PSO, n = 20), lesional allergic contact dermatitis skin (ACD, n = 10), and healthy skin (HS, n = 10). (b) IL-36 gene expression in lesional skin of 10 psoriasis patients before and after 16 weeks of anti-TNF-α treatment. (c) IL-36γ serum levels in HC (n = 10), PSO (n = 10), and ACD (n = 10) subjects. Supernatants from healthy skin organ culture treated with TNF-α (20ng/ml) for 24 hours were used as positive controls (CTRL+). (d) IL-36γ gene expression in peripheral blood mononuclear cells (PBMCs) of HC (n = 5), PSO (n = 5), and ACD (n = 5) subjects. Data are displayed as mean ± standard deviation. *P < 0.05, **P < 0.01. 18S rRNA, 18S ribosomal RNA; ACD, allergic contact dermatitis; CTRL+, positive control; HS, healthy skin; PBMC, peripheral blood mononuclear cell; PSO, psoriatic skin; Ra, receptor antagonist; TNF, tumor necrosis factor.
infiltration, cell death, and proliferation (Mutamba et al., 2012; Tortola et al., 2012). We speculate that in an ex vivo ACD non lesional skin system, IL-36\(\gamma\) produced by KCs promotes skin T-cell infiltration, amplifying cutaneous damage with an increase in IL-36\(\gamma\). The expression of IL-36\(\gamma\) is highly complex, and it may have distinct functions depending on the context in which it is expressed. It was reported that IL-36\(\gamma\) acts as an alarmin, being up-regulated by toll-like receptor ligand expressed in response to the innate immune system activation and having a central position in proinflammatory pathways at the interface between innate and adaptive immunity (Lian et al., 2012). Furthermore, IL-36\(\gamma\) induces the production of several proinflammatory cytokines, including IL-12, IL-6, TNF-\(\alpha\), and IL-23 (Carrier et al., 2011) involved in both psoriasis and ACD. All these evidences support our hypothesis that IL-36\(\gamma\) could represent a marker for skin inflammation and not a specific one for psoriasis.

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CONFLICT OF INTEREST
The authors state no conflict of interest.

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Figure 2. Inflammatory cytokine gene expression in peripheral blood mononuclear cells (PBMCs) from whole study population and ex vivo ACD non lesional skin assay. (a) IL-36\(\gamma\) gene expression in PBMCs of 5 HC, 5 PSO, and 5 nickel-ACD subjects stimulated with concanavalin A (ConcA, 5 \(\mu\)g/ml), toxic shock syndrome toxin (TSST-1, 100ng/ml), and nickel sulfate (NiSO\(_4\), 10\(^{-4}\) mol/L) for 24 hours. (b) IL-22 gene expression in PBMCs of 5 HC, 5 PSO, and 5 nickel-ACD subjects stimulated with TSST-1 (100ng/ml), NiSO\(_4\) (10\(^{-4}\) mol/L), and recombinant IL-36Ra (rIL-36Ra, 1.25 \(\mu\)g/ml) for 24 hours. (c) IL-36\(\gamma\) gene expression in healthy skin (HS, \(n = 5\)) and in non lesional (NLes) nickel-ACD skin (\(n = 5\)) stimulated with 30 mg of NiSO\(_4\) (5% in petrolatum). PBMCs isolated from the same subjects were incubated in the system with or without NiSO\(_4\). (d) IL-36\(\gamma\), TNF-\(\alpha\), and IL-1\(\alpha\) gene expression in PBMCs isolated from nickel-ACD subjects (\(n = 5\)) stimulated with NiSO\(_4\) (10\(^{-4}\) mol/L) and in PBMCs collected from ex vivo ACD NLes skin assay. Data are displayed as mean ± standard deviation. --, without treatment; +, with treatment. * \(P < 0.05\), ** \(P < 0.01\), *** \(P < 0.001\). 18S rRNA, 18S ribosomal RNA; ACD, allergic contact dermatitis; ConcA, concanavalin A; HC, healthy control; HS, healthy skin; PBMC, peripheral blood mononuclear cell; NLes, non lesional; PSO, psoriatic skin; rIL-36Ra, recombinant IL-36Ra; TNF, tumor necrosis factor; TSST-1, toxic shock syndrome toxin.

SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at www.jidonline.org, and at http://dx.doi.org/10.1016/j.jid.2016.03.020.

REFERENCES
Papain Activates Human Mast Cells to Release Proinflammatory Mediators via its Enzymatic Activity


TO THE EDITOR

IgE-independent mast cell (MC) activation is an important event that can take place even in an allergy setting. This kind of activation can be elicited by endogenous substances, such as major basic protein 1 and nerve growth factor secreted from activated eosinophils (Ben-Zimra et al., 2013; Solomon et al., 1998), and by other mediators produced by different cells in late and chronic stages of allergic inflammation. Other MC IgE-independent activators are exogenous substances such as codeine, opiates, bacteria, and selected cosmetic ingredients that were demonstrated to activate human skin MCs (Mathelier-Fusade, 2006). Papain, a food derivative and a cosmetic ingredient, belonging to the cysteine proteases family (Stack et al., 2011) is an important allergen and is particularly interesting for its effect in the skin. It induces itch without evident histamine release probably due to direct nerve fiber activation (Reddy and Lerner, 2010). In mice, papain impairs the skin barrier by degrading tight junction proteins and increasing the number of MCs (Stremnitzer et al., 2015). It also induces an IgG1 response and MC degranulation (Chambers et al., 1998). However, there are no reports on papain’s direct activity on human MCs. Therefore, in this work, we have investigated whether papain could directly induce human MC activation. We demonstrated that papain can activate human MCs partly via protease-activated receptor 2 (PAR-2) and its enzymatic activity, and we defined the signal transduction of this event.

To first assess whether papain could activate human MCs in situ, papain was added to foreskin pieces (1 hour). Toluidine blue staining of sections from these experiments revealed several degranulated MCs (Figure 1a and b). Moreover, papain induced in a dose-dependent fashion tryptase release from the skin pieces (Figure 1c) indicating a direct activity on skin MCs in an ex vivo complex setting. Next, to confirm the direct effect of papain on