Trem-1 is not crucial in psoriasiform imiquimod-induced skin inflammation in mice

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Abbreviations: DC, dendritic cell; IFN-γ, interferon-gamma; IL-17, interleukin-17; IL-10, interleukin-10; IMQ, imiquimod; Th, T helper lymphocyte; TLR, Toll-like receptor; Trem, triggering receptor expressed on myeloid cells.

Key words: imiquimod – psoriasis – Trem-1

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Background
Psoriasis is a chronic inflammatory skin disorder involving dendritic cells (DCs), macrophages, neutrophils and T cells with a predominant Th1 and Th17 response leading to dermo-epidermal inflammation and hyperkeratosis (1). Trem (triggering receptor expressed on myeloid cells) proteins are a family of cell surface receptors expressed on neutrophils, monocytes/macrophages (2) and some subset of epithelial cells (s1, s2). Engagement of Trem, after association with DAP12, has been shown to stimulate the production of pro-inflammatory chemokines such as IL-8, CCL-2 and CCL-7, as well as inducing rapid neutrophil degranulation (3). Activation of Trem-1 in the presence of TLR-2, TLR-4 or TLR-7 ligands amplifies the production of TNF-α and IL-1β (s3) (4). Trem-1 has been mainly studied during septic shock but is also critical during aseptic inflammation, in its both acute (e.g. pancreatitis) (s4) and chronic form (e.g. rheumatoid arthritis) (5). It was recently shown in human that Trem-1 expression was increased in active psoriatic skin lesions in comparison with treated psoriatic skin lesions and healthy skin (6). TREM-1 blocking of ex vivo psoriatic human DCs that express TREM-1 reduced Th17 response leading to the hypothesis that TREM-1 could be a potential target in psoriasis (6). The imiquimod (IMQ)-induced psoriasis-like skin inflammation mouse model (7) mirrors many immunological aspects of human psoriasis and is widely used to decipher psoriasis mechanisms (8). Topical application of Aldara® (containing IMQ and isostearic acid) induces a psoriatic skin inflammation involving inflammasome activation (mainly TLR-7).

Question addressed
In this study, we investigated the clinical and immunological effects of Trem-1 genetic invalidation in the IMQ-induced model of psoriasis.

Experimental design
Trem-1 knockout (Trem-1−/−) (s5) adult male C57BL/6 and Trem-1+/− littermates were treated with IMQ cream (62.5 mg, 5%, Aldara®; MEDA Pharma, Paris, France) or vaseline for 6 days on the shaved back or the left ear (7). Severity of skin inflammation was scored daily using a cumulative score (0–12) of erythema, scaling and thickening (0–4) and on ear measured thickness (mm, Digital Caliper micrometer).

Results
As shown in Figure 1, IMQ-treated Trem-1−/− mice did not have different clinical psoriasis severity than Trem-1+/− littermates (Fig. 1a, b). To test whether Aldara® topical treatment induced the expression of Trem-1, we evaluated the expression of Trem-1 on spleen mononuclear cells in the three groups of mice (Trem-1−/− mice treated with vaseline, Trem-1+/− mice treated with Aldara®, Trem-1−/− mice treated with Aldara®) using flow cytometry (Appendix S1). Aldara® topical application increased by two-fold the spleen size and the splenocyte number (data not shown). As shown in Figure 1c, Aldara® treatment induced a three- to fourfold increase in Trem-1 expression compared with vaseline. To further evaluate disease severity, the degree of skin inflammation of the back skin was assessed using histopathology (H&E-stained 5-μm skin section) and CD3+ T-cell immunohistochemistry (Appendix S1). Epidermal thickness (Figure S1a), cellular infiltrate, hyperkeratosis, parakeratosis, micro-abscesses (data not shown) and CD3+ T-cell infiltration (Figure S1b) were not statistically different between Trem-1−/− and Trem-1+/− littermates at day 6 after IMQ treatment. Despite any clinical and histopathological effect, we investigated the effect of Trem-1 on the splenic and skin cytokine profile in this model. IMQ treatment induced a significant production of interleukin-17A (IL-17A),...
Figure 1. Trem-1 has no significant effect on the severity of psoriasis in the imiquimod (IMQ)-induced model. Shaved back skin or ears of Trem-1+/+ and Trem-1−/− mice (> 6 mice each group) were topically treated with an IMQ-containing cream or vaseline for 6 consecutive days. (a) Representative phenotypical presentation of mouse back skin after 6 days of IMQ treatment (left panel); daily cumulative score (erythema plus scaling plus thickening) as a measure of the severity of inflammation (scale 0–12) (right graph). (b) Representative phenotypical presentation of ear swelling after 6 days of IMQ treatment (left panel); daily ear thickness (right graph). (c) Induction of Trem-1 expression in the splenic mononuclear cells after 6 days of IMQ treatment. Values indicated in the dot plot represent the frequencies of Trem-1-expressing mononuclear cells as measured by flow cytometry. Histograms show the MFI of Trem-1 expression in the different groups of mice. Indicated values represent means (± SEM) and are representative of 3 experiments.

Conclusions

In this study, we did not evidence any significant clinical effect of Trem-1 invalidation on the course of psoriasisiform IMQ-induced skin inflammation in mice. However, Trem-1 was strongly induced in the spleen of IMQ-treated Trem-1+/+ mice (Figure S1 b), suggesting that Aldara® may activate the Trem-1 pathway probably via interconnected TLR pathways. As Trem-3 may have overlapping functions with Trem-1, it could be interesting to investigate the intensity of psoriasis in the Trem-1−/−/ Trem-3−/− mouse model (9). In our experiments, the frequency of both IFN-γ- and IL-10-producing CD4+ T cells was unexpectedly increased in Trem-1−/− IMQ-treated mice, suggesting a possible role of Trem-1 in the regulation of IMQ-induced inflammation. IFN-γ has pro-inflammatory effects, whereas IL-10 has anti-inflammatory effects, which may explain the absence of clinical impacts of these cytokine changes. Even if Trem-1 has mainly pro-inflammatory effects, it has been shown in human monocytes that Trem-1 may downregulate the expression of interleukin-12 and interleukin-23 after TLR-4 activation (s6). The IMQ-induced model of psoriasis is a simple acute model of inflammation (8), which does not rule out a possible role of Trem-1 in other mouse models of psoriasis or in human psoriasis. Other psoriasis mouse models involving other immunological pathways could be investigated for the role of Trem-1 (use of Trem-1 blocking agents); transgenic psoriatic disease models such as CD18 hypomorphic mice (s7) and inducible JunB/c-Jun double KO mice (s8); and a xenotransplant model where healthy skin from a psoriatic patient is grafted onto immunodeficient mice (s9). To conclude, even if no obvious effect of Trem-1 genetic invalidation on psoriasisiform IMQ-induced skin inflammation in mice has been shown, further studies should be conducted to test TREM-1 as a potential target in human psoriasis.

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Author contribution

Ji, EH, LZ, LL, SC, AdM, JDB and HAO performed the research; Ji, EH, Mbat, HIB, ABe, SG, JDB and HAO designed the research study; Ji, LZ, LL, HIB, SC, MBat, AdM, HIB, SG, ABe, JDB and HAO contributed essential reagents or tools; Ji, EH, LZ, LL, SC, MBat, AdM, HIB, SC, MBag, ABe, SG, JDB and HAO analysed the data. Ji, EH, MBag, ABe, SG, JDB and HAO wrote the manuscript.

Conflict of interest

The authors state no conflict of interest.

Supporting Information

Additional supporting data may be found in the supplementary information of this article.

Figure S1: Trem-1 has no significant histological effect on the severity of psoriasis in the imiquimod (IMQ)-induced model. (a) Representative skin sections of the back after 6 days of IMQ treatment (H&E staining, original magnifications X 200) (left pictures); measure of epidermal thickness of skin sections (right graph). (b) Representative immunostaining of CD3+ T cells of skin sections after 6 days of IMQ treatment (left pictures); the numbers of CD3+ T cells were counted (right graph).

Figure S2: Trem-1 has no major effect on cytokine transcription induced by imiquimod (IMQ)-containing cream treatment. Levels of interleukin-17 (IL-17), interferon-gamma (INF-γ), interleukin-10 (IL-10) and interleukin-23 (IL-23) in the skin as measured by quantitative PCR. Indicated values represent means (± SEM) and are representative of 3 experiments. Significant differences between sample means are indicated: *P < 0.05; **P < 0.001.

Appendix S1 Methods.
Decreased expression of activin A receptor 1C may result in \( \text{Ca}^{2+} \)-induced aberrant skin hypersensitivity

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Background

Sensitive skin is a hyperactive skin condition characterized by exaggerated sensory symptoms in response to internal stimulants and external irritants such as cosmetics (1). Recently, we showed that sensitive skin is associated with dysfunction of muscle contraction and metabolic homeostasis including adiponectin deficiency (2,3). Our previous microarray data set (GEO series accession number GSE48506) revealed that the expression of various signalling pathway-regulated genes including activin A receptor 1C (ACVR1C) were markedly down-regulated in sensitive skin compared with that in non-sensitive skin (3). ACVR1C, also known as activin receptor-like kinase 7 (ALK7), is a type I serine/threonine kinase receptor for the transforming growth factor (TGF)-\( \beta \) superfamily. TGF-\( \beta \) superfamily signalling plays an important role in the regulation of cell growth, differentiation, apoptosis and immunosuppression. In particular, ACVR1C is involved in the regulation of metabolic homeostasis in pancreas and adipose tissues, as a preferred receptor for various ligands such as activin AB, activin B and Nodal (4).

Question addressed

Here, we aim to elucidate the roles of ACVR1C and its ligand with regard to sensitive skin.

Experimental design

See supplementary methods for details.

The expression of ACVR1C in sensitive and non-sensitive skin was validated using quantitative PCR, Western blot and immunofluorescence. Healthy volunteers who perceived their skin to be ‘sensitive’ or ‘non-sensitive’ were classified based on self-assessment questionnaires and 10% lactic acid stinging test \( (n = 5/\text{group}) \), as previously described (3). Then, we obtained skin samples from face or buttock. This study was approved by the local Institutional Review Board, and all subjects provided written informed consent. The study was conducted according to the Declaration of Helsinki.

Results

Consistent with previous microarray data, ACVR1C mRNA and protein were significantly decreased in sensitive skin compared with non-sensitive skin (Fig. 1a–c). In human facial skin, striated muscle fibres are found in the reticular dermis and subcutis (5), in addition to smooth muscles (arrector pili). ACVR1C is strongly expressed in non-sensitive skin, particularly in muscle tissues (Fig. 1c).

Sensitive skin is characterized by pathologic nociception due to the activation of transient receptor potential cation channel subfamily V member 1 (TRPV1) associated with aberrant muscle contraction (3,6). More specifically, our prior study suggested that decreased synthesis of ATP and enhanced intracellular acidity caused by abnormal regulation of genes involved in muscle contraction, acidic homeostasis and energy metabolism lead to aberrant muscle contraction and skin sensitivity in human (3). Muscle contraction is a complex process which is precisely regulated by interactions between contractile proteins such as actin, myosin, titin and tropomyosin, calcium ions and their transport systems, as well as ATP. Calcium ion balance is an

References