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Loss of keratinocyte Mcpip1 abruptly activates the IL-23/Th17 and Stat3 pathways in skin inflammation

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To the Editor,

Monocyte Chemotactic Protein-1-Induced Protein 1 (MCPIP1; also known as Regnase-1 and encoded by the *ZC3H12A* gene) is a negative regulator of inflammatory processes [1,2]. MCPIP1 possesses a conserved CCCH-type zinc finger domain and PilT N-terminus (PIN) domain with RNase properties [1,3]. It recognizes specific stem-loop structures in 3′ untranslated regions and mediates the destabilization of transcripts encoding, for example, pro-inflammatory cytokines and transcription factors [1–3]. Previous studies indicated that MCPIP1 is both transcriptionally and translationally activated in the human psoriatic epidermis [4,5]. It was further discovered that Mcpip1 deficiency contributes to enhanced sensitivity to imiquimod (IMQ). In particular, heterozygous Mcpip1$^{-/+}$ mice developed IMQ-induced psoriasiform inflammation faster than control mice [6]. This phenotype was not attributed to changes in the mRNA expression of interleukin-23 (IL-23)/T-helper 17 (Th17) cytokines but rather was due to enhanced IL-17 receptor (IL-17r) signalling [6]. In addition, a recent study by Takaishi et al. showed that the loss of Mcpip1 expression in keratinocytes led to an exaggerated response to IMQ stimulation, similar to what was shown in heterozygous Mcpip1$^{-/+}$ mice. The authors indicated proved the involvement of IL-36r signalling in the development of the psoriasis-like phenotype. However, Mcpip1^{EKO}:IL-36r^{KO} mice were only partially protected against IMQ-induced skin inflammation [7]. Based on those reports, we hypothesized that additional factors drive the enhanced sensitivity of keratinocyte-specific Mcpip1-deficient (Mcpip1^{EKO}) mice to IMQ-driven skin inflammation.

To address this question, we utilized a classic model of psoriasis-like skin inflammation induced by IMQ [8]. We previously showed that upon ageing, Mcpip1^{EKO} mice develop spontaneous skin pathology, most likely as a result of elevated inflammatory signalling [9]. Keratinocyte-specific ablation of Mcpip1 upregulated the transcriptional expression of genes related to inflammation and keratinocyte differentiation, such as *Il36a*, *Il36g*, *S100a8*, *S1000a9*, *Defb3*, *Sprr2d* and *Sprr2h* [7,9]. To induce psoriasis-like inflammation, we applied IMQ-containing cream to the shaved backs of control and Mcpip1^{EKO} mice for four consecutive days (Fig. 1A). Topical IMQ treatment resulted in the accelerated development of psoriasis-like skin symptoms in Mcpip1^{EKO} mice, as indicated by an abrupt increase in the Psoriasis Area and Severity Index (PASI) score (Fig. 1B and Suppl. Fig. S1A). Histological analyses performed 96 h after IMQ application confirmed the psoriasis-specific pathology of the skin, which was characterized by epidermal skin thickening (acanthosis), hyperkeratosis, and the retention of nuclei within corneocytes (parakeratosis) (Fig. 1C). The major phenotypical

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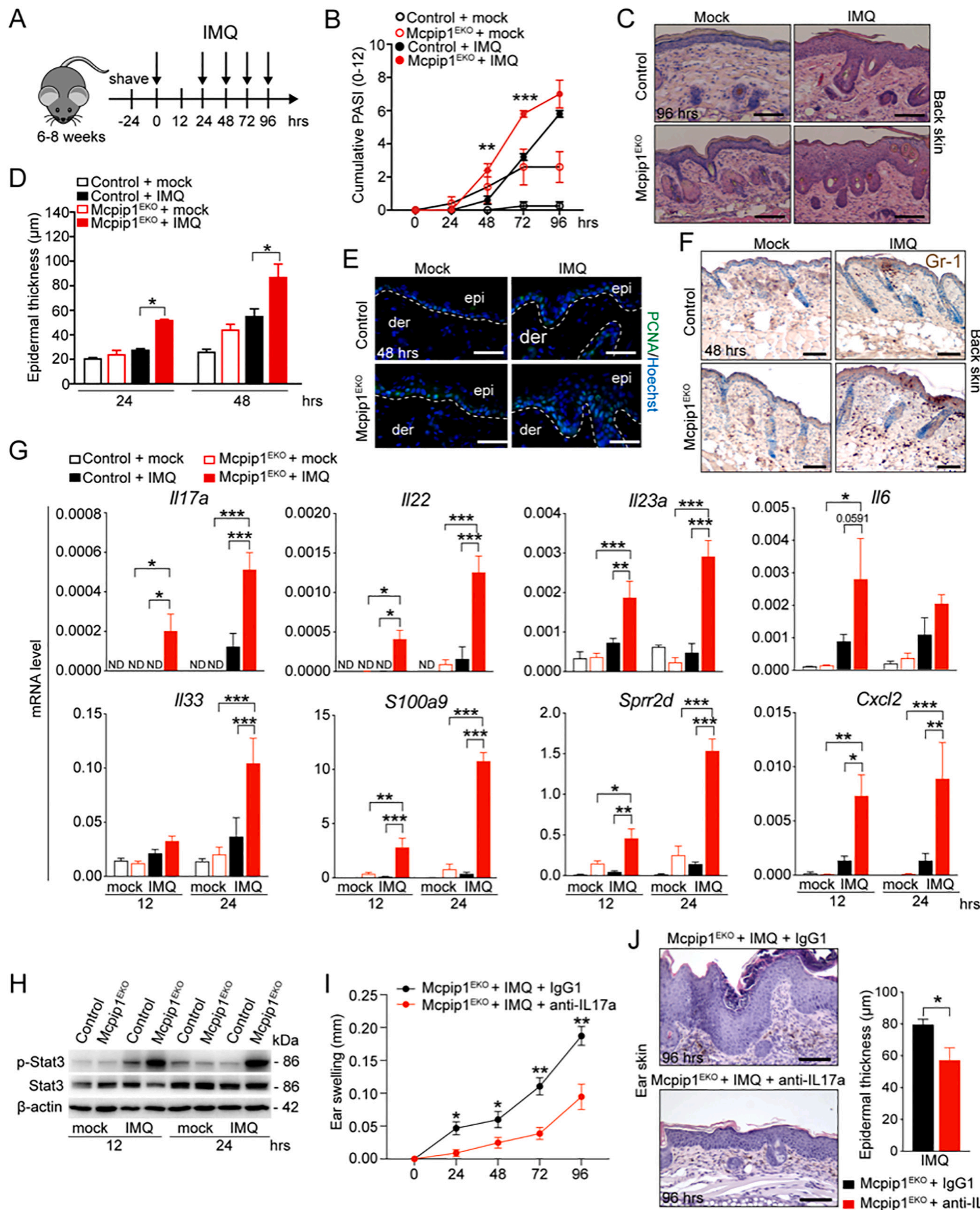
changes observed in psoriatic skin develop via abnormal proliferation and differentiation of keratinocytes. To investigate these processes in our model, skin sections were stained for keratin 14 (Krt14), a marker of basal keratinocytes, and Krt10, which is expressed in suprabasal differentiating epidermal cells. Immunohistochemistry showed a reduction in keratinocytes expressing Krt10 with the simultaneous increased expression of Krt14 in suprabasal epidermal layers, indicating the abnormal differentiation and accelerated proliferation of Mcpip1^{EKO}



(caption on next page)

Fig. 1. Mcpip1 deficiency promotes psoriasiform dermatitis. (A) A schematic diagram of a 96-h time course of IMQ application. (B) Disease severity during IMQ treatment; $n = 5$ (3 independent experiments). Clinical scores for disease severity were calculated daily using a scoring system based on the clinical Psoriasis Area and Severity Index (PASI); $n = 5$. For statistical analysis, IMQ-treated Mcpip1^{EKO} mice were compared to IMQ-treated control mice. (C) Microscopic analysis of H&*E*-stained dorsal skin sections at 96 h. Scale bar: 100 μm. (D) Microscopic measurement of the *epi*dermal thickness of back skin at 24 and 48 h; $n = 3$. (E) Immunostaining for PCNA at 48 h. Scale bar: 50 μm. (F) Immunohistochemical staining of Gr1$^+$ cells at 48 h. Scale bar: 100 μm. (G) Total RNA was isolated from control and Mcpip1^{EKO} mock/IMQ-treated mouse skin. qRT-PCR analysis of *Il17a*, *Il22*, *Il23a*, *Il6*, *Il33*, *S100a9*, *Sprr2d and Cxcl2* expression levels was carried out. *Ef2* was used as a reference gene; $n = 4$. (H) Representative Western blot among three independent experiments to detect p-Stat3 and total Stat3 from whole-skin protein lysates. β-Actin was used as a loading control. (I-J) Mcpip1^{EKO} mice ears were treated with IMQ for 96 h in the presence of IL-17a or isotype control antibodies (I) Ear swelling; n = 5–6 (3 independent experiments). (J) Microscopic analysis of H&E-stained ear sections at 96 h and microscopic measurement of the ear epidermal thickness; n = 5–6. Data are shown as the mean and SEM. The dashed line indicates the basal membrane. IMQ – imiquimod; epi – epidermis; der – dermis; hf – hair follicle; ND – not detected by qRT-PCR (a value of zero was given for statistics). Two-way ANOVA or unpaired *t*-test (I and J) was used to calculate *P*-values. Asterisks indicate statistically significant differences. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

keratinocytes compared to control keratinocytes following 96 h of treatment with IMQ (Suppl. Fig. S1B). We also noticed a mild proliferation/differentiation disturbance phenotype in mock-treated Mcpip1^{EKO} mouse skin (Fig. 1C and Suppl. Fig. S1B).

Subsequently, we examined the kinetics with which histological features of psoriasis develop in our system. Comparison of the histological morphology of IMQ-treated Mcpip1^{EKO} and control mouse skin revealed that Mcpip1 loss led to a more dynamic increase in epidermal thickness upon IMQ application. Compared to the control mouse epidermis, the Mcpip1^{EKO} mouse epidermis was almost 2-fold thicker in response to treatment with IMQ for only 24 h and remained significantly elevated after 48 h (Fig. 1D). Accordingly, in both groups of mice, increased proliferation, assessed by staining for proliferating cell nuclear antigen (PCNA), was observed 48 h after IMQ application; however, this effect was much more pronounced in Mcpip1^{EKO} mice (Fig. 1E). At this time point, we also observed the increased infiltration of Gr1$^+$ cells (neutrophils and monocytes) to IMQ-treated Mcpip1^{EKO} skin. It was previously shown that neutrophils accumulated in the skin of haploinsufficient Mcpip1$^{-/+}$ mice following IMQ treatment [6]. In this study, we report that specific ablation of keratinocyte Mcpip1 is sufficient to promote Gr1$^+$ cells infiltration into the skin during the early stages of IMQ-induced inflammation (Fig. 1F).

Having characterized the major histological features of skin inflammation induced by IMQ, we attempted to explore molecular factors that contribute to the accelerated development of psoriasiform inflammation in Mcpip1^{EKO} mice. Several psoriasis-associated factors are commonly described, among which the IL-23/Th17 axis plays a crucial role [10]. IL-23 promotes the development of IL-17- and IL-22-producing Th17 cells. Psoriatic lesions are characterized by elevated expression of IL-23 and increased numbers of Th17 cells. Both IL-17 and IL-23 cytokines enhance dermal acanthosis, neutrophil recruitment and the infiltration of IL-22- and IL-17-producing cells into lesional skin [11]. In a recent study, Takaishi and colleagues did not observe significant differences between Mcpip1^{EKO} mice and control mice in terms of IMQ-induced IL-23/Th17 axis mRNA levels after 72 h of IMQ treatment [7], similar to what was previously shown in heterozygous Mcpip1$^{-/+}$ mice by Monin et al. [6]. In agreement with these observations, we did not observe significant differences in the relative expression levels of *Il17a*, *Il22* and *Il23a* transcripts after 96 h of IMQ stimulation (Suppl. Fig. S2A).

We hypothesized that the mechanisms that trigger psoriasis-like inflammation are very abruptly activated in Mcpip1^{EKO} mice. In addition to measuring gene expression levels at 96 h, we performed analyses at earlier time points following IMQ application. To that end, samples for qRT-PCR analyses were collected at 12 and 24 h following initial IMQ application (Fig. 1A and G). We observed that the IMQ-induced mRNA expression of a plethora of pro-inflammatory mediators in Mcpip1^{EKO} skin was already altered within the first 24 h. In particular, transcript levels of the major IL-23/Th17 cytokines IL-17a, IL-22 and IL-23 in the whole skin of Mcpip1^{EKO} mice were significantly increased after 12 and 24 h following IMQ application (Fig. 1G). Consistently, transcriptional expression of the *Il6*, *Il33*, *S100a9*, *Sprr2d* and *Cxcl2* genes, which are related to inflammation, keratinocyte differentiation and antimicrobial defence and associated with the psoriatic phenotype, was abruptly induced in Mcpip1^{EKO} mouse skin, with kinetics similar to those of the activation of genes encoding IL-23/Th17 cytokines (Fig. 1G). The expression patterns of selected mRNAs explain the abrupt development of the psoriasis-like phenotype in Mcpip1^{EKO} mice.

Next, we investigated the activation of Stat3 signalling, which plays an important role in Th17 cell biology and has been implicated in the pathogenesis of psoriasis and other autoimmune inflammatory diseases, in our model of psoriasis-like inflammation [12,13]. The transcriptional regulator STAT3 was identified as an acute phase response factor activated by IL-6 [11]. STAT3 is also regulated by other factors, including IL-17, IL-22 and IL-23 [14,15]. Our studies indicate that the treatment of both control and Mcpip1^{EKO} mouse skin with IMQ for 96 h led to the activation of Stat3 (Suppl. Fig. S2B). In addition, detailed analysis of the kinetics of Stat3 phosphorylation revealed that in Mcpip1^{EKO} mice, Stat3 was activated 12 and 24 h after IMQ application with simultaneous transcriptional activation of pro-psoriatic cytokines (Fig. 1H and Suppl. Fig. S3A).

Altogether, in this study, we showed that depletion of Mcpip1 in keratinocytes led to a highly aggravated skin inflammation phenotype upon the IMQ-mediated induction of psoriasis-like skin inflammation. IMQ treatment greatly accelerated the induction of pro-psoriatic gene expression in Mcpip1^{EKO} mice, which was associated with the appearance of histological inflammatory features and neutrophil infiltration. In particular, we showed that the loss of keratinocyte Mcpip1 led to enhanced proliferation and reduced differentiation and neutrophil accumulation in response to stimulation with IMQ.

To the best of our knowledge, this is the first study to utilize such short-term application (12–24 h) of IMQ. Using this approach, we identified psoriasis-associated molecular factors that were rapidly activated in the skin of Mcpip1^{EKO} mice. In particular, we showed that IL-23/Th17 cytokine mRNA levels varied significantly between control and Mcpip1^{EKO} mouse skin at very early time points upon stimulation with IMQ. In line with this, the level of IL-23 protein was elevated in Mcpip1^{EKO} skin treated with IMQ for 24 h (Suppl. Fig. S3B). Consistently with this observation, the IL-17a blockade significantly weakened the IMQ-induced inflammatory phenotype of Mcpip1^{EKO} mice (Fig. 1I and J) suggesting that the inflammatory phenotype must, at least partially, depend on IL-23/Th17 pathway. Thus, our results may explain why inhibiting IL-36 signalling only partially protected Mcpip1^{EKO} mice against IMQ-induced skin inflammation. Most likely, the IL-23/Th17, IL-33 and IL-36 axes are together involved in the rapid development of psoriasiform dermatitis in mice with keratinocyte-specific ablation of Mcpip1. Our work expands current knowledge of the role of the Mcpip1 protein in modulating psoriasis-like inflammation. It also indicates that factors controlling the posttranscriptional regulation of inflammatory gene expression may contribute to skin diseases and act as potential therapeutic targets.

CRediT authorship contribution statement

Agata Lichawska-Cieslar: Conceptualization, Methodology, Investigation, Writing - original draft, Visualization. **Piotr Konieczny:**

Conceptualization, Methodology, Investigation, Writing - original draft, Visualization. **Weronika Szukala:** Investigation. **Wim Declercq:** Conceptualization, Supervision, Writing - review & editing. **Mingui Fu:** Methodology, Resources, Writing - review & editing. **Jolanta Jura:** Project administration, Funding acquisition, Conceptualization, Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbamcr.2020.118866.

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