INTRODUCTION

Psoriasis is a systemic chronic autoimmune skin disease characterized by the overproliferation of keratinocytes.[1–4] In psoriasis, keratinocyte differentiation is hampered, as cells do not die via apoptosis, resulting in a thicker epidermis in patients.[2,4] Chronic systemic...
inflammation in psoriasis manifests in the skin and the joints. Recent studies show that psoriasis is driven by the IL23/Th17 axis, together with IL6. To date, there is no curative treatment for psoriasis.

The poly(ADP-ribose) polymerase (PARPs or ARTDs) family consists of 17 members. PARP1 is responsible for the majority of the PARP activity in cells and tissues. Classical PARP enzymes, such as PARP1, can cleave NAD⁺ to ADP-ribose and nicotinamide and use ADP-ribose to synthesize ADP-ribose polymers on acceptor proteins, changing the biological activity of the acceptor proteins. PARP activation was shown to be a key regulator of a switch between cell survival, apoptosis and necrosis. In fact, PARP inhibition can protect against cell death.

PARP1 is a well-known mediator of inflammatory pathologies. PARP1 impacts the immune system at multiple points, including the differentiation and maturation of immune cells. PARP1 is a positive modulator of several pro-inflammatory transcription factors. Thus, the absence of PARP1 is anti-inflammatory in Th1- and Th2-mediated pathologies. Importantly, PARP1 activation is also crucial in skin inflammatory processes and it seems that PARP inhibition is anti-inflammatory in humans, as well.

In this study, we aimed to assess whether PARP1 can influence Th17-mediated pathologies, such as psoriasis.

2 | METHODS

2.1 | Chemicals

All chemicals were from Sigma-Aldrich unless otherwise stated.

2.2 | Cell culture

Cellular studies were performed on HPV-KER keratinocytes. For the generation of an immortalized keratinocyte cell line, normal human adult keratinocytes (NHEK) were obtained from a healthy individual undergoing routine plastic surgery at our department and transfected with the pCMV vector containing the HPV16/E6 oncogene. A stable cell line was established by continuous culturing over 70 passages before the start of our experiments.

The HPV-KER cells were cultured in Keratinocyte-SFM medium supplied with prequalified human recombinant Epidermal Growth Factor 1 and Bovine Pituitary Extract (Gibco, Thermo Fisher Scientific). The medium was supplemented with 1% L-glutamine and 1% Penicillin/Streptomycin antibiotics (Lanza). Cells were kept under standard laboratory conditions (37°C in a humidified atmosphere containing 5% (v/v) CO₂).

Cells were treated with imiquimod (IMQ—100 µM), PARP inhibitors (Olaparib—1 µM, Rucaparib—1 µM, PJ34—3 µM), TRPV1 inhibitor (AMG9810—100 nM) or an inhibitor for classical PKCs (Gö6976—2 µM) for 3 hours.

2.3 | Animal studies

We elicited psoriasiform skin lesions in littermate PARP1⁺/⁻ and PARP1⁻/⁻ male mice using imiquimod, as described in (study registration No. 15/2016/DEMÁB). Hair was shaved off the back of male mice in a 1 × 1 cm area, and mice were treated daily with 100 µl Aldara cream or vehicle. The severity of the psoriasiform lesions was scored daily by two experienced, blinded dermatologists on a scale from 0 to 4, where 0 denotes no symptoms and 4 denotes the most severe symptoms. On day 5, 2 hours prior to sacrifice, mice received 5-bromo-2-deoxyuridine (BrDU, 100 mg/kg i.p.), then mice were sacrificed, and skin biopsies were taken for histology examination.

Mice were subsequently bred at the animal facility of the University of Debrecen. No more than six mice were housed in each cage (standard block shape 365 × 207 × 140 mm, surface 530 cm²; 1284 L Eurostandard Type II. L from Techniplast) with Lignocel Select Fine (J. Rettenmaier und Söhne) as bedding. Mice had paper tubes to enrich their environment. Dark/light cycle was 12 h, and temperature 22 ± 1°C. Cages were changed once a week, on the same day. Mice had ad libitum access to food and water (sterilized tap water). The animal facility was overseen by a veterinarian. Group sizes are indicated in the figure captions.

2.4 | Histology

Immunohistochemistry was performed similarly to using antibodies against 5-bromo-2-deoxyuridine (BrDU; 115855960001; Roche), involucrin (PRB-140C; Covance) and IL6 (ab6672; Abcam). PARP1 immunohistochemistry was performed as described in using Abcam ab227244 antibody (1:100). For terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL), we used the commercially available ApopTaq kit (Millipore). We used visual scores (0-3) by two blinded researcher to quantify histology results.

2.5 | Assessment of cellular proliferation

Cellular proliferation was assessed in sulphorhodamine assay was performed as briefly. HPV-KER cells were seeded in 96-well plates and were treated with the chemicals indicated for 3 hours with a prior 30 minutes treatment with the PARP inhibitors. Cells were fixed and stained with SRB. SRB was liberated and measured by spectrophotometry.

2.6 | Cellular assays

mRNA expression was assessed in RT-qPCR reactions. RNA was prepared and commercially available Taqman assays
2.7 | Statistical analysis

Statistical tests are indicated in figure captions. $P < .05$ is considered statistically significant.

3 | RESULTS

3.1 | The deletion of PARP1 exacerbates imiquimod-induced psoriasis

Surprisingly, the disease was more severe macroscopically in the PARP1−/− than in the PARP1+/+ mice (Figure 1A-B), although prior studies identified PARP1 as a pro-inflammatory protein. In accordance, the thickening of the epidermis was more pronounced in the PARP1−/− than in the PARP1+/+ mice (Figure 1C). Proliferation was similar in the epidermis of the imiquimod-treated PARP1+/+ and PARP1−/− mice, as shown by the number of BrDU-positive cells (Figure 1D). However, the number...
of TUNEL (terminal deoxynucleotidytransferase dUTP nick end labelling) positive cells, indicative of cell death, was drastically reduced in the PARP1−/− mice compared with the PARP1+/+ mice (Figure 1E). Consistent with the increased severity of the disease, the expression of the differentiation marker involucrin decreased in the PARP1−/− mice compared with the PARP1+/+ mice (Figure 2A). Finally, the expression of disease-promoting interleukin, IL6, increased in the epidermis of the PARP1−/− mice compared with the PARP1+/+ mice (Figure 2B).

We assessed the expression of PARP1 in psoriatic lesions and control dry skin in human samples from similar anatomical locations and in the IMQ-induced murine model. PARP1 expression decreased in human psoriatic lesions (Figure 2C). We observed similar decreases in PARP1 expression in human psoriatic lesions in datasets[17,18] from NCBI GEO. We also detected lower PARP1 expression in IMQ-induced psoriasis, which did not reach the level of significance (Figure 2D). In another dataset[19] that compared animal models of psoriasis, we observed that decreases in PARP1 expression are a common feature of most murine models investigated.

We continued our investigation by assessing the response of HPV-KER cells to IMQ and PARP inhibition. PARP1 is responsible for 80%-85% of total cellular PARP activity[20,21]; therefore, it was a valid technique to apply PARP inhibitors (Olaparib, Rucaparib and PJ34) in cellular models to block PARP1. The lack of PARP activity was assessed by Western blotting (Figure 3A). PARP inhibitor treatment alone or in combination with IMQ increased proliferation as measured in SRB assays (Figure 3B) recapitulating our previous in vivo observations. We assessed the expression of cytokines relevant for psoriasis (IL6, IL1β and IL8). IMQ treatment increased the mRNA expression of these cytokines, moreover, when applied in combination with PARP inhibitors cytokine expression was further exacerbated (Figure 3C). The expression of IL17 and IL23 followed the same pattern; however, readouts were not significant.

**FIGURE 2** Genetic deletion of PARP1 inhibits keratinocyte differentiation and IL6 expression. (A-B) 100 µl Aldara cream (containing imiquimod, IMQ) or 100 µl vehicle was applied onto the shaved back skin of PARP1+/+ and PARP1−/− male mice (5 months of age) (PARP1+/+ CTL n = 10, PARP1−/− CTL n = 10, PARP1+/+ IMQ n = 11, PARP1−/− IMQ n = 9) daily for 5 days. Upon sacrifice, skin biopsies were taken and subjected to histological processing and examination. On histological sections, immunocytochemistry was performed for (A) involucrin (PRB-140C, Covance, Princeton, NJ, USA) and (B) IL6 (ab6672, Abcam, Cambridge, UK). (C-D) In (C) dry skin (n = 5) and psoriatic lesions, as well as in murine samples from the study (PARP1+/+ CTL n = 10, PARP1−/− CTL n = 10, PARP1+/+ IMQ n = 11, PARP1−/− IMQ n = 9), we performed PARP1 immunohistochemistry (Abcam ab227244 1:100). WC - PARP1+/+ CTL, PC - PARP1−/− CTL, WP - PARP1+/+ IMQ, PP - PARP1−/− IMQ. In all panels, chi-square tests were used to calculate significance that was re-tested using Fisher’s exact test. * denotes significant difference between PARP1+/+ IMQ and PARP1−/− IMQ groups at P < .05; # denotes significant difference between CTL and IMQ groups. Scale bar equals 100 µm.
3.2 | PARP1-mediated effects depend on TRPV1 activity

TRPV1 was shown to be a positive mediator of psoriasis\(^{22}\); therefore, we assessed whether the inhibition of TRPV1 could reverse the joint action of imiquimod and PARP inhibitors. We performed sulforhodamine B assays, in which TRPV1 inhibition prevented the induction of total protein content upon imiquimod and PARP inhibitor co-treatment (Figure 4A) suggesting an involvement of TRPV1 in the keratinocyte hyperproliferation. As TRPV1 is a calcium channel, we tested how calcium chelation interferes with PARP inhibitor treatment. PARP1 activity was shown to be mediated by classical PKC enzymes\(^{23}\). Therefore, we assessed whether the pharmacological inhibition of classical protein kinase C enzymes by Gö6976; however, Gö6976 did not influence cellular proliferation (Figure 4B).

4 | CONCLUSIONS

In this study, we show that the genetic deletion of PARP1 exacerbates an experimental model of psoriasis, the Th17 dependency of which was demonstrated previously.\(^{12}\) There is a wide array of literature showing that PARP1 has pro-inflammatory properties in Th1- and Th2-mediated pathologies\(^{7,24,25}\); hence, the pharmacological or genetic inhibition of PARP1 is anti-inflammatory in these conditions.\(^{16}\) Therefore, the exacerbation of experimental psoriasis in the absence of PARP1 was surprising. In the PARP1 knockout mice, the immune component of the disease was dysregulated, as highlighted by the increased IL6 staining in the epidermis of PARP1\(^{-/-}\) mice.

The deletion of PARP1 derailed keratinocyte cell death differentiation, as indicated by the decreased number of TUNEL-positive
cells and decreased epidermal involucrin staining in the PARP1−/− mice. PARP1 has intricate roles in regulating cell differentiation and has a well-documented role in the regulation of cell death.[6] The phenotype we observed, on one hand, corresponds to the known cell death-promoting features of PARP1.[6] While on the other, there are studies suggesting that PARP activation can limit the self-renewal capacity of stem cells, which may also contribute to the phenotype we observed.[29‒32]

As we pointed out earlier, transient receptor potential vanilloid 1 (TRPV1) is a positive regulator of psoriasis[22,33,34] and we showed that the pharmacological blockade of TRPV1, but not the blockade of classical PKCs, can suppress the PARP inhibitor-elicited exacerbation of keratinocyte proliferation.

This study is the first to show an opposing role for PARP1 in a Th17-mediated pathology compared with Th1 or Th2 (skin) pathologies.[6‒8,27,35] Moreover, we provide evidence that PARP1 expression is decreased in the lesions of psoriasis patients, suggesting that our findings can be translated to humans.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS
BK and PB conceptualized the study; BK, HC, AS1, MS, DA, JM and PB performed experiments; BK, AS1 and PB evaluated histology; GM performed PARP1 histology; BK, LV, AS2 and PB wrote the manuscript. AS1—Annamária Szödényi. AS2—Andrea Szegedi.

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DATA AVAILABILITY STATEMENT
Primary data are available at https://figshare.com/s/5a2f4b033a bb49b9434a (https://doi.org/10.6084/m9.figshare.7791407).

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