Our data show that one-third of patients with PD have autoantibodies to Col XVII that also bind to TH^+ neurons. The lack of reactivity of PD autoantibodies with skin suggests the autoantibodies develop from neuronal Col XVII. The subset of patients with neurologic disease that develop BP likely result from epitope spreading to regions of Col XVII that are pathogenic in skin. A key question that remains is whether Col XVII autoantibodies have a detrimental effect on PD or are otherwise predictive of disease onset/outcomes. Finally, these data suggest loss of tolerance to neuronal Col XVII may contribute to the risk for pemphigoid.

CONFLICT OF INTEREST

The authors state no conflicts of interest.

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SUPPLEMENTARY MATERIAL

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Human β-Defensin 3 and Its Mouse Ortholog Murine β-Defensin 14 Activate Langerhans Cells and Exacerbate Psoriasis-Like Skin Inflammation in Mice

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TO THE EDITOR

Genetic variations in the proinflammatory cytokine IL-23 are associated with psoriasis, a common, inflammatory skin disorder (Nair et al., 2009). IL-23 is primarily produced by dendritic cells (DCs) and plays a pathogenic role in psoriasis by directing the development of T helper type 17 cells (Di Cesare et al., 2009). Langerhans cells (LCs), the main DC subtype in the epidermis, can participate in both immunity and the induction of tolerance (Igyarto et al., 2011; Seneschal et al., 2012), yet their role in psoriasis remains unclear. Mouse models of disease have yielded contradictory results (Glitzner et al., 2014; Wohn et al., 2013; Yoshiki et al., 2014). However, the migration of LCs is impaired in human disease, and it was suggested that the inflammatory environment of psoriasis may affect their function (Cumberbatch et al., 2006). This study sought to clarify the role of LC role in disease with specific focus on the production of IL-23.

Punch biopsies (6 mm) were obtained from psoriasis patients and healthy

Abbreviations: DC, dendritic cell; HBD3, human β-defensin 3; LC, Langerhans cell; MBD14, murine β-defensin 14; moLC, monocyte-derived Langerhans cell

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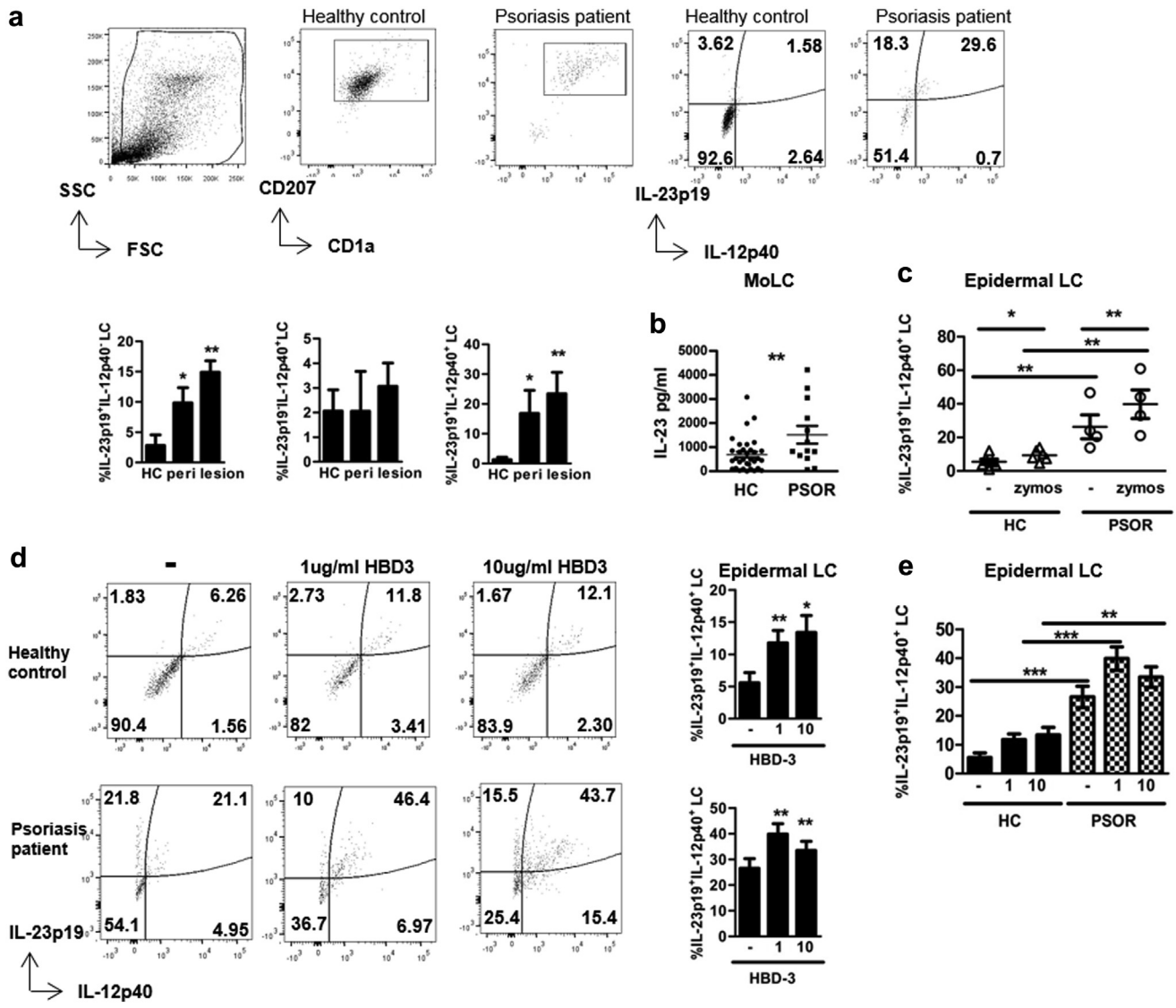

Figure 1. IL-23 production by Langerhans cells (LCs) is enhanced in psoriasis. (**a**) Epidermal suspensions from healthy controls and psoriasis patients were treated with monensin for 6 hours and IL-23 expression by LCs (live, $CD45^+CD1a^+CD207$ cells) was analyzed by flow cytometry. Results are given as mean ± standard error of the mean for 6−8 donors. (**b**) Monocyte-derived LCs from psoriasis patients (PSOR) and controls (HC) were stimulated with zymosan (10 μg/ml). After 24 hours, the concentration of IL-23 was determined by ELISA. (**c, d**) Epidermal LCs from psoriasis patients (PSOR) and healthy controls (HC) were treated with (**c**) zymosan or (**d**) human β-defensin 3 (HBD3) for 24 hours. Monensin was added for the final 12 hours, and the expression of IL-23 by LCs was determined by flow cytometry. Results are given as mean ± standard error of the mean for 4−8 donors. Representative plots are pregated on live $CD45^+CD207^+CD1a^+$ LCs. (**e**) IL-23 expression by epidermal LCs from psoriasis patients (PSOR) and healthy controls (HC) (as in **d**). Results are given as mean ± standard error of the mean for 6−8 donors, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

controls after written informed patient consent and ethical approval were obtained from St. Vincent's Ethics and Medical Research Committee. LCs in epidermal suspensions were identified as live single $CD45^+CD1a^+CD207^+$ cells (Figure 1a; see Supplementary Materials online). A significantly higher percentage of epidermal LCs expressed IL-23p19 or coexpressed p40 and p19 in psoriatic lesional and perilesional biopsies compared with LCs from healthy epidermis ($P < 0.01$, $P < 0.05$; Figure 1a). There was no significant difference in the expression of IL-12p40 only (Figure 1a). These results suggest that under inflammatory conditions, LCs are capable of producing proinflammatory cytokines that promote T helper type 17 cell development.

Since monocyte-derived LCs (moLCs) play an important role in repopulating the epidermis during inflammation-induced migration (Ginhoux et al., 2006), we next examined IL-23 production by moLCs. LC differentiation was confirmed by examining the expression of CD1a, E-cadherin, and CD207 (see Supplementary Figure S1 online). moLCs from psoriasis patients were found to express significantly higher levels of IL-23 upon zymosan stimulation compared with moLCs from healthy controls ($P < 0.01$; Figure 1b). Furthermore, although zymosan induced a small but significant expression of IL-23 by epidermal LCs from healthy controls ($P < 0.05$; Figure 1c), it induced significantly more IL-23 by epidermal LCs from psoriasis patients, suggesting that factors associated with the inflammatory microenvironment of psoriasis

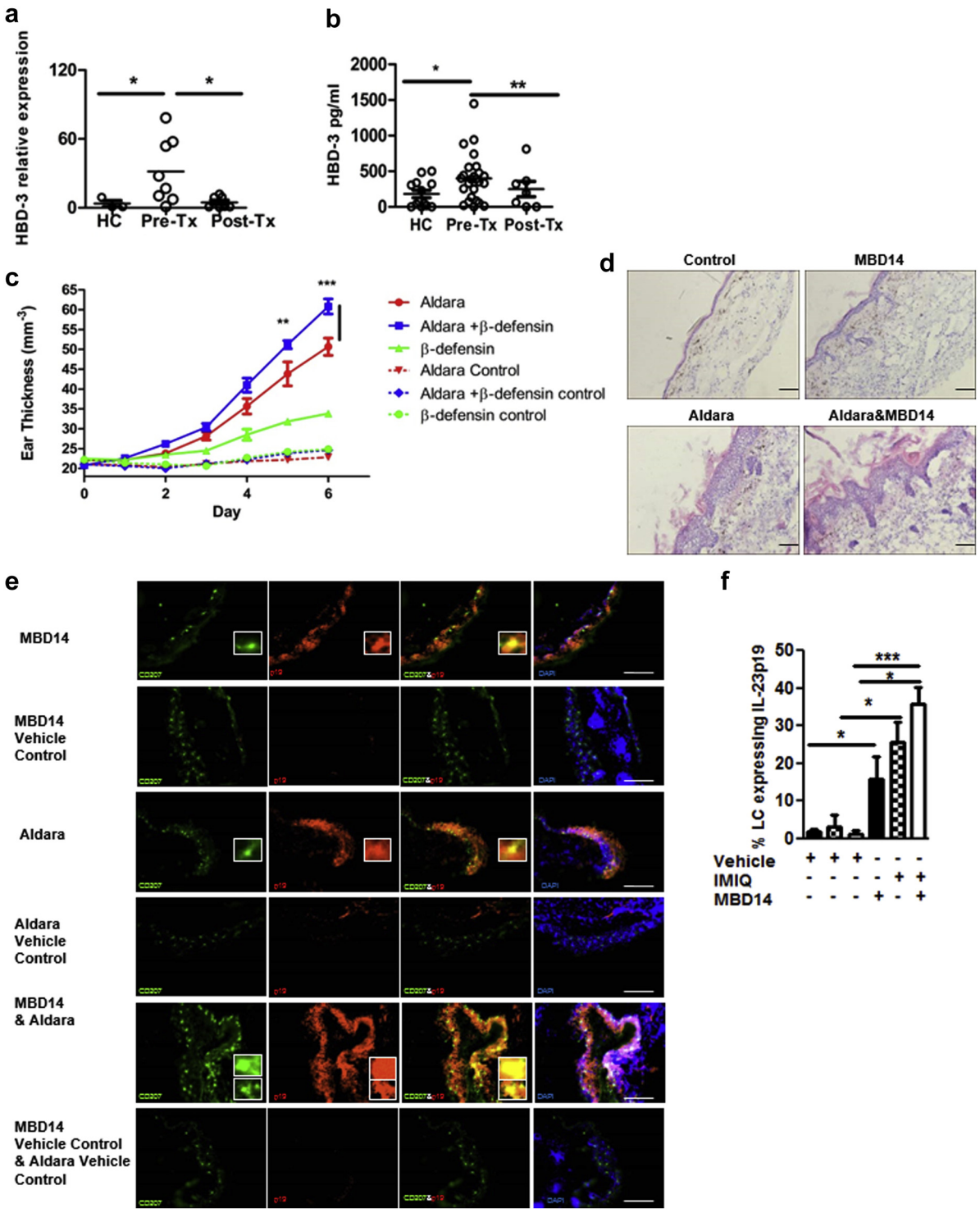

Figure 2. Human β-defensin 3 (HBD3) is enhanced in psoriasis and its ortholog mouse β-defensin 14 (MBD14) exacerbates psoriasis-like inflammation in mice, which is associated with an increase in IL-23p19 expression by Langerhans cells (LCs). (**a**) HBD3 mRNA and (**b**) protein expression was determined in the (a) skin (n = 8) and (b) serum (n = 25) of psoriasis patients before and after (n = 6–7) treatment and from control skin (HC; n = 3) and serum (HC; n = 13) by reverse transcriptase-PCR and ELISA, respectively. (**c**) Psoriasis-like skin disease was induced in C57BL/6 mice with topical application of Aldara cream; intradermal administration of MBD14 (1 μg in phosphate buffered saline) and topical application of Aldara; or intradermal administration of MBD14 (1 μg in phosphate buffered saline) only for 7 days. Matched control ears received vehicle treatment. (**c**) Ear thickness was measured using a thickness gauge (Hitec) before initiation of the experiment on day 0 and every day for 7 days. Clinical thickness of control (vehicle) and treated ears are shown as mean score ± standard error of the mean (n = 5–6). (**d, e**) Ears from mice were removed and frozen in optimal cutting temperature compound. (**d**) Psoriasis severity and (**e**) expression of IL-23p19 (red) by LCs (green) counterstained with 4′,6-diamidino-2-phenylindole (DAPI; blue) were determined using hematoxylin and eosin staining and three-color immunofluorescence, respectively. Bar = 100 μm. (**d**) Representative control image displayed. (**e**) High magnification inset (magnification ×60) from displayed images are depicted. Magnification ×40 also depicted for MBD14&Aldara group (bottom inset). (**f**) Percentage of IL-23$^+$ LCs shown as mean score ± standard error of the mean. n = 5–6, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

cooperate with zymosan to promote IL-23 expression. We next sought to determine the mechanism driving IL-23 expression by LCs in psoriasis. Antimicrobial peptides in complex with self-nucleotides have been implicated in the pathogenesis of psoriasis through the production of proinflammatory cytokines by plasmacytoid (Lande et al., 2007) and myeloid DC (Ganguly et al., 2009). Human β-defensin 3 (HBD3), a small antimicrobial peptide, is chemotactic for immune cells and is enhanced in psoriasis (Lande et al., 2015). HBD3 in complex with self-DNA (Tewary et al., 2013) was shown to induce IFN-α production by plasmacytoid DC (Tewary et al., 2013) and in cooperation with other antimicrobial peptides can break tolerance to self-DNA (Lande et al., 2015). Other studies have shown that HBD3 alone enhances the expression of costimulatory molecules on LCs (Ferris et al., 2013), suggesting that HBD3 may modulate antigen-presenting cells in psoriasis. Given its effects on LCs, we examined whether HBD3 plays a role in the dysregulation of LCs in psoriasis. HBD3 induced IL-23 and enhanced zymosan-induced IL-23 production by healthy moLCs (see Supplementary Figure S2 online). Moreover, HBD3 alone induced the production of IL-23 by epidermal LCs from psoriasis patients and healthy controls ($P < 0.01$; Figure 1d). However, HBD3-induced IL-23 production was significantly increased in psoriasis patients compared with healthy controls ($P < 0.01$; Figure 1e), suggesting that increased IL-23 in psoriasis is a result of HBD3 and the psoriatic environment (possibly endogenous HBD3). These results indicate that HBD3 may play a pathogenic role in psoriasis.

We next examined the expression of HBD3 in psoriasis. The mRNA expression of HBD3 was increased in psoriasis skin during active disease and decreased upon clearance of psoriasis with UVB treatment to levels comparable with that of healthy controls ($P < 0.05$; Figure 2a). Similar results were obtained in the serum ($P < 0.01$; $P < 0.05$, Figure 2b). To confirm the pathogenic role of HBD3 in psoriasis, we next examined the effect of HBD3 in a murine model of disease. Psoriasis-like skin inflammation was induced in C57BL/6 mice with imiquimod formulated in a commercially available cream (Aldara, MEDA Pharmaceuticals, Dublin, Ireland); Aldara and murine β-defensin 14 (MBD14), a murine ortholog of HBD3 (Hinrichsen et al., 2008); or MBD14 only for 7 days. As expected, application of Aldara cream resulted in psoriasis-like skin inflammation as measured by increased epidermal thickness (Figure 2c), acanthosis, desquamation, parakeratosis, and infiltration (Figure 2d). However, MBD14 exacerbated epidermal ear thickness and psoriasis-like skin inflammation ($P < 0.01$, $P < 0.001$; Figure 2c and d), and MBD14 alone induced a mild disease (Figure 2c and d). To investigate the role of LCs in this model, we examined IL-23p19 expression by LCs by immunofluorescence (see Supplementary Materials). IL-23p19 was strongly expressed in ears that received Aldara or Aldara plus MBD14 (Figure 2e) but was absent in control skin (Figure 2e). From 25% to 35% of LCs expressed IL-23p19 in epidermis from inflamed skin (Figure 2f) induced with Aldara (mean 25.64%) or Aldara plus MBD14 (mean 35.4%). In accordance with a milder clinical phenotype, 15% of LCs expressed IL-23p19 in epidermis from mice treated with MBD14 only (Figure 2f). These results suggest that MBD14 exacerbates psoriasis-like skin inflammation and alone induces a mild disease in mice, which is associated with an increased expression of IL-23p19 by LCs. This strongly suggests that HBD3 plays a pathogenic role in psoriasis by promoting the expression of IL-23 by LCs and that LCs likely act as sources of IL-23 in disease. Recent studies have demonstrated that conventional langerin-negative DC produce IL-23 to induce psoriasis-like inflammation in mice and suggest that LCs are dispensable for the induction of disease (Wohn et al., 2013). In contrast, it was shown that LCs are a major DC source of IL-23 during psoriasis-like inflammation and are essential for disease induction (Yoshiki et al., 2014). It is worth noting that each of these studies differed in their precise methods of disease induction or LC ablation but established IL-23 as a key player in driving psoriasis-like inflammation. Although it is unclear whether LCs play a role in the induction of psoriasis-like inflammation in mice, our results demonstrate that LCs are capable of producing IL-23 in disease. Thus, it may be that although LCs are not necessary for the initial induction of disease, activation of LCs by HBD3 sustains and amplifies an established inflammatory response. Regardless, our study clearly demonstrates that HBD3 drives the production of IL-23 by LCs in psoriasis, which contributes to the pathogenesis of disease.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

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Short Wavelength Visible Light Suppresses Innate Immunity-Related Responses by Modulating Protein *S*-Nitrosylation in Keratinocytes

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TO THE EDITOR

The solar radiation spectrum reaching the earth's surface consists mostly of ultraviolet A (UVA), visible, and infrared light. UVA irradiation has clinical applications for patients requiring local immune-suppression therapy (Weatherhead et al., 2012). However, UV radiation is carcinogenic and is not recommended for long-term treatment (Kunisada et al., 2007). Thus, visible light-based therapies with less harmful effects on human skin are desirable. For example, blue light (400–450 nm) irradiation is used therapeutically to treat severe atopic dermatitis (Becker et al., 2011), 632.8-nm light enhances cell proliferation, and red light (550–670 nm) accelerates epidermal permeability barrier recovery after disruption (Denda and Fuziwara, 2008; Hu et al., 2007). However, the mechanisms underlying various effects of visible light are not clear.

Human skin exhibits innate immune responses, such as epithelial defense via antimicrobial peptides (AMPs), and the release of proinflammatory cytokines involved in the recognition of microbes via toll-like receptors (TLRs) (Gallo and Nakatsuji, 2011; Meyer et al., 2007). Thus, we investigated the effects of visible light on innate immunity. The survival rate of normal human epithelial keratinocytes (NHEKs) was not affected by visible light irradiation (Supplementary Figure S1 online). Violet or blue light downregulated the mRNA expression levels of AMPs after one or three exposures (Figure 1a, b and Supplementary Figure S2a online). By contrast, UV irradiation upregulated AMPs, except for *LL-37* (Supplementary Figure S2b). The expression levels of AMPs (human beta-defensins (HBD-1, -3)) and proinflammatory cytokines (RANTES, MCP-1, and IL-8) decreased after violet light irradiation in 3D skin (Supplementary Figure S2c). This violet light-induced downregulation of AMPs affected bacterial survival. Bacteria grew better in a violet light-irradiated NHEK-conditioned medium than in a control NHEK-conditioned medium, irrespective of polyinosinic-polycytidylic acid (poly I:C), which amplifies innate immune responses (Supplementary Figure S3 online).

We examined whether violet light influences TLR ligand-induced responses. Poly I:C, but not Pam3, lipopolysaccharide, or CpG, increased *HBD-1, -2,* and *-3* simultaneously, which was significantly decreased by violet light. Flagellin-induced increases in *HBD-2* and *S100A7* were reduced by violet light (Figure 1c). Poly I:C-induced increases in proinflammatory cytokines were decreased by violet light in NHEKs and 3D models (Supplementary Figure S4a online). Unlike violet light, red light did not affect the poly I:C-induced augmentation of AMPs and proinflammatory cytokines (Supplementary Figure S4b).

When TLRs are activated via ligands, the NF-κB signaling cascade is activated and proinflammatory cytokines are

Abbreviations: AMPs, antimicrobial peptides; HBD, human beta-defensins; NHEKs, normal human epithelial keratinocytes; poly I:C, polyinosinic-polycytidylic acid; SNO, S-nitrosylated; TLRs, Toll-like receptors; UVA, ultraviolet A

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