Phospholipase C epsilon mediates cytokine cascade induced by acute disruption of epidermal permeability barrier in mice

Jing Zhang a,b,1, Jiangmei Wu b,1, Mengke Sun b,1, Shuchang Zhang b, Junkai Huang b, Maoqiang Man c, Lizhi Hu a,b,*

a Immunology Department, Key Laboratory of Immune Microenvironment and Disease (Ministry of Education), Tianjin Medical University, Tianjin, 300070, China
b Department of Pathobiology and Immunology, Basic Medical College, Tianjin Medical University, Tianjin, 300070, China
c Dermatology Services, University of California San Francisco, San Francisco, CA, 94121, USA

A R T I C L E   I N F O
Keywords:
IL-22
PLCε
Skin inflammation
Barrier function
Psoriasis

A B S T R A C T
Disruption of epidermal barrier is an important trigger in abnormal cutaneous inflammation. Phospholipase C epsilon (PLCε), a Ras/Rap1 effector, is essential for regulating cytokines production in different types of skin inflammation. Our previous studies have demonstrated that elevated expression of PLCε participates in the psoriasis-like inflammation in PLCε overexpressing transgenic mouse model, while the reduction in PLCε expression attenuates inflammatory responses in either TPA- or DNFB-induced cutaneous inflammation. Here, we determined the role of PLCε in cutaneous inflammation induced by acute abrogation of epidermal permeability barrier. In comparison to wild type controls, PLCε KO mice exhibited reduced ear swelling and infiltration of granulocytes after tape-stripping. Moreover, expression levels of pro-inflammatory cytokines (IL-1α, IL-1β), chemokines (CXCL-1, CXCL-2, CCL20), and antimicrobial peptides (S100 proteins, MBD3) were lower in PLCε-deficient versus wild type mice. Likewise, expression levels of cytokines and chemokines were also lower in PLCε-deficient keratinocytes and fibroblasts following IL-22 stimulation in vitro. Furthermore, knockdown of PLCε with its siRNA decreased expression of IL-1α, CCL20, and S100 proteins, and MBD3 in HEK cultures. Collectively, these results suggested that PLCε-mediated cytokine cascade induced by acute barrier disruption. IL-22 is likely the upstream of PLCε-mediated cytokine cascade following acute barrier disruption.

1. Introduction

Interleukin (IL)-22, a member of IL-10 superfamily, is involved in the modulation of inflammatory response, immunosurveillance and homeostasis at multiple barrier surfaces [1]. A growing number of evidence suggests that IL-22 participates in skin homeostasis and pathogenesis of various skin diseases, such as psoriasis, atopic dermatitis, and skin cancer [2,3]. The interaction of IL-22 and its receptor IL-22R/IL-10R2 contributes to skin inflammation and immune barrier through modulating the expression of numerous cytokines (IL-1β, TNF-α, IL-6, etc.), chemokines (CXCL-1, CXCL-2, CXCL-5, CXCL-8, etc.), and antimicrobial peptides (S100A family genes, beta-defensin family genes, etc.) [4–6]. These molecules are responsible for recruitment of immune cells to inflammatory site, and innate immune response to pathogen invasion [7]. An aberrant cascade of proinflammatory factors may directly lead to continuous inflammation process, and can contribute to the development of series of inflammatory diseases, including psoriasis. The receptor of IL-22 is highly expressed in both fibroblasts and keratinocytes, and the signal primarily goes by phosphorylation of activating signal transducer and activator of transcription 3 (STAT3) through JAK/STAT pathway [7].

As a member of the phosphoinositide-specific PLC family that is

Abbreviations: PLCε, phospholipase C epsilon; TNF, tumor necrosis factor; TGF, transforming growth factor; K1, keratin 1; FLG, filaggrin; LOR, loricrin; IVL, involucrin; SPT1, serine palmitoyltransferase 1; TLR2, toll like receptor 2; SHH, sonic hedgehog; BMP4, bone morphogenetic protein 4; LHX2, LIM homeobox 2; SOX9, SRY-box 9; K15, keratin 15; CXCL, chemokine (C-X-C motif) ligand; CCL20, chemokine (C-C motif) ligand 20; MBD, murine beta defensin; STAT3, transducer and activator of transcription 3; HEK, human epidermal keratinocytes; PMA, Phorbol-12-myristate-13-acetate.

* Corresponding author. Immunology Department, Key Laboratory of Immune Microenvironment and Disease (Ministry of Education), Tianjin Medical University, Tianjin, 300070, China.
E-mail address: lizhihu@tmu.edu.cn (L. Hu).

1 These authors contributed equally to this work.

https://doi.org/10.1016/j.bbrep.2020.100869
Received 16 November 2020; Accepted 23 November 2020
2405-5808/© 2020 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
regulated by Ras and Rap small GTPase, phospholipase C epsilon (PLCe) mediates diverse signals in the development of skin inflammation. Our previous studies have shown that PLCe has similar regulatory role to IL-22 in inducing cutaneous inflammation. For example, overexpression of epidermal PLCe induces psoriasis-like skin lesion, and increases cytokine expression, including IL-22, while PLCe deficiency decreases inflammatory responses, including decreased a panel of cytokine expression, but not IL-22, and neutrophil infiltration in murine dermatitis models [8–10]. Being interested in the relationship of IL-22 and PLCe, and their role in inflammation, we investigated the role of PLCe in cutaneous inflammatory response to acute barrier disruption and the link between IL-22 and PLCe.

2. Material and methods

2.1. Animals and acute barrier disruption

Mice with inactivated PLCe allele were established and validated as described previously [11,12]. For acute disruption of the skin barrier, 8-week old PLCe KO and wild type mice were tape-striped for 3 times with a cellophane tape. Afterward skin samples of 2 cm² were obtained at the indicated time points for qRT-PCR. According to our prior studies, a minimum of three animals are required to achieve statistical comparison.

All the animals were housed in a pathogen-free animal facility with temperature of 22 ± 1 °C, relative humidity of 50 ± 1% and a light/dark cycle of 12/12 h. All animals had free access to regular solid chow and drinking water. All animal studies (including the mice euthanasia procedure) were done in compliance with the regulations and guidelines of Tianjin Medical University institutional animal care and conducted according to the AAALAC and the IACUC guidelines.

2.2. Histology

The skin samples were obtained from the ears 24 and 48 h after tape-stripping, and fixed in 4% PFA for H&E staining, as described previously [13].

2.3. Isolation, culture, and stimulation of mouse fibroblasts and keratinocytes

Primary dermal fibroblasts and epidermal keratinocytes were isolated from new born PLCe KO and wild type mice, and cultured as described previously [9]. Both fibroblasts and keratinocytes were stimulated with IL-22 (50 ng/ml, 582-ML/CF, R&D Systems) for 0, 3, 6, 12, 24 h, followed by harvest of cells for qRT-PCR analysis.

2.4. Culture, silencing and stimulation of human epidermal keratinocytes

Primary human epidermal keratinocytes (HEK) were purchased from Lifeline Cell Technology in America and cultured in Defined Keratinocyte-SFM supplemented with growth factors (Lifeline, MD, USA). HEK were transfected with PLCe siRNA (Santa Cruz Biotechnology, CA, USA) using previously described approach [13]. Cells were stimulated with medium containing IL-22 (10 ng/ml, 782-82-ML/CF, R&D Systems).

2.5. qRT-PCR analysis

Total RNA was isolated from excised tissue and cells using Trizol reagent (Invitrogen, Carlsbad, CA) and RNeasy Mini kit (Qiagen, Düsseldorf, Germany), following the manufacturer’s instructions. cDNA synthesis was performed with TaqMan Reverse Transcription kit (Applied Biosystems, CA, USA). The qRT-PCR was performed with 7500 Real-Time PCR system. Relative mRNA levels of each sample were determined by the ΔΔCt method with GAPDH. Primers are listed in Supplementary Table 1.

2.6. Statistical analyses

Data were presented as means ± SD. One-tailed Student’s t-test was performed for determination of P values. P value < 0.05 was considered statistically significant.

3. Results

3.1. Ablation of PLCe decreases skin inflammation induced by acute disruption of epidermal permeability barrier

Our prior study showed that PLCe ablation suppresses skin inflammatory response in various dermatitis models [8,9,14]. To explore whether PLCe is also involved in the inflammatory response to disruption of the skin barrier, first we compared the inflammatory reactions by H&E staining. As shown in Fig. 1, the ears of PLCe KO mice displayed moderate swelling and less granulocyte infiltration compared to wild type controls both 24 h and 48 h after tape-stripping. We further examined the inflammatory cytokine, proliferation and differentiation factor, antimicrobial peptide levels in the whole skin of PLCe KO and CON mice after acute disruption of epidermal permeability barrier (Fig. 2 a and b). In parallel, expression levels of mRNA for a number of inflammatory cytokines and chemokines were lower in the whole skin of PLCe KO than in that of wild type mice after acute disruption of epidermal permeability barrier. Notably, expression levels of IL-22 was significantly increased in both PLCe KO and wild type with a PLCe-independent manner after barrier disruption (Fig. 2b). These results indicate that PLCe deficiency reduces inflammatory response to inflammation induced by disruption of epidermal permeability barrier.

3.2. IL-22 increases expression levels of cytokines and chemokines in fibroblast and keratinocyte cultures

Because IL-22 is a well-known upstream regulator of cytokine cascade in inflammatory response, we assessed next whether PLCe deficiency-associated reduction in inflammatory response is mediated by IL-22 [1]. Previous study showed that both fibroblasts and keratinocytes are cellular targets for IL-22 [15]. We first evaluated the impact of IL-22 on inflammation in fibroblasts from PLCe KO and wild type mouse skin in vitro. Our results showed that IL-22 markedly increased expression levels of mRNA for cytokines, chemokines and antimicrobial peptides in fibroblasts from wild type mice, while minimally increased in fibroblasts from PLCe KO mice (Fig. 3a). These results suggest a crucial role of PLCe in IL-22-induced inflammation in fibroblasts.

Epidermal keratinocytes can also produce and release cytokines in response to various stimuli. Next, we investigated the effect of PLCe in IL-22-induced inflammation in keratinocytes. As shown in Fig. 3b, treatment of keratinocytes with IL-22 increased expression levels of mRNA for cytokines, chemokines and antimicrobial peptide starting as early as 3 h after addition of IL-22 to control keratinocytes. In contrast, IL-22 only induced a minimal increase in expression levels of these mRNA at 12 and 24 h in PLCe KO keratinocytes. These results indicate a requirement of PLCe for IL-22-induced inflammation in keratinocytes.

To further confirm the role of PLCe in IL-22-induced inflammation, we assessed expression levels of mRNA for cytokines and chemokines in HEK transfected with PLCe siRNA, followed IL-22 treatment. As shown in Fig. 3c, expression levels of mRNA for IL-1α, IL-8, and CCL20 were significantly higher in the controls than in HEK-treated with PLCe siRNA 3 h after IL-22 stimulation. In addition, mRNA levels of S100A8, S100A12, and beta-defensin were also higher in the controls than in HEK-treated with PLCe siRNA 12 h after IL-22 stimulation. These results implicated that IL-22-induced productions of pro-inflammatory cytokines, chemokines, and antimicrobial peptides are mediated by PLCe in keratinocytes.
4. Discussion

Regulatory role of epidermal permeability barrier in keratinocyte proliferation, differentiation and antimicrobial peptide production is well appreciated [15]. Disruption of epidermal permeability barrier can provoke cutaneous inflammation, predisposing to the development of...
some inflammatory dermatoses, such as psoriasis, contact dermatitis and cutaneous infections [17–19]. However, the underlying mechanisms how barrier disruption induces cutaneous inflammation are unknown. The present study demonstrated that acute disruption of epidermal permeability barrier dramatically increased expression levels of several cytokines, chemokines and antimicrobial peptides in wild type mice, but only IL-22 was increased in PLCε KO mice. Interestingly, silence of PLCε with its siRNA also lowered expression levels of cytokine and chemokines following stimulation of keratinocytes with IL-22. Collectively, the present study demonstrates that 1) PLCε mediates, at least in part, the induction of cutaneous inflammation by barrier disruption; 2) IL-22 is likely the upstream of cytokine cascades in cutaneous inflammation induced by barrier disruption.

Although the mechanisms by which barrier disruption induces IL-22 expression are unknown, the results of the present study suggest that the pathogenic role of IL-22 in psoriasis could be mediated by PLCε. Previous studies showed that expression levels of IL-22 is higher in psoriatic lesion and the blood of psoriatic patients [5,20]. Either overexpression of IL-22 or administration of IL-22 to the skin induces psoriatic lesion [21]. Conversely, mice with IL-22 deficiency or anti-IL-22 antibody treatment do not develop psoriasis-like lesion after imiquimod treatments [22,23]. Mice with overexpression of PLCε also develop psoriasis-like inflammation phenotypes, driven by aberrant expression of proinflammatory molecules represented by IL-22 and IL-23 [10]. Coupling the results of the present study, IL-22/PLCε signaling pathway is possibly involved in the pathogenesis of psoriasis. Because psoriasis vulgaris preferably occurs on the body sites vulnerable to superficial trauma, e.g., disruption of barrier by scratch or abrasion, and the Köbner phenomenon, we hypothesize that disruption of epidermal permeability barrier induces the release of IL-22, resulting in cytokine cascade mediated by PLCε, consequently leading to the development of psoriasis (Fig. 3d).

This study still exists some limitations. First, as we found the possibility of IL-22 directly activating PLCε in keratinocytes, we still need to show the engagement of the IL-22R and the subsequent signal transduction, such as Ras/Rap and STAT3 signaling, required for activation of PLCε and downstream cytokine cascade induction. Second, a mouse model simulating psoriatic inflammation in PLCε KO mice is required for further evidence to confirm the participation of PLCε and IL-22 in psoriasis.

5. Conclusions

The present study demonstrate that PLCε is also required for the development of cutaneous inflammation induced by acute disruption of epidermal permeability barrier, and the regulator role of IL-22 in cutaneous inflammation is mediated, at least in part, by PLCε, suggesting a pathogenic role of IL-22/PLCε signaling pathway in psoriasis.

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.

![Fig. 3. IL-22 induces cytokine cascades in fibroblasts and keratinocytes mediated by PLCε. The mRNA levels of cytokine, chemokines, and antimicrobial peptides induced by IL-22 (50 ng/ml) for 0, 3, 6, 12, 24 h in primary cultured fibroblasts (a) and keratinocytes (b) from newborn PLCε KO and CON mice. (c) HEK were transfected with PLCε siRNA (siRNA) and control siRNA (CON). The mRNA levels of cytokines, chemokines and antimicrobial peptides in HEK 0, 3, 12, 24 h after treatment of IL-22 (10 ng/ml) were detected. The data are presented as mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001. (d) A model figure of IL-22-induced inflammatory reaction after skin barrier disruption. Abrogation of epidermal permeability barrier leads to acute inflammation response initiated by inflammatory factors including IL-22. We hypothesize that the release of IL-22 probably activates PLCε and induces downstream cytokine cascade, which consequently elicits the new psoriatic lesion.](image-url)
Acknowledgements

This work was supported by National Natural Science Foundation of China (grant numbers: NSFC81972962, 81573075 and 81301360, to L.H) and Key Laboratory of Immune Microenvironment and Disease (Ministry of Education).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2020.100869.

References

[1] C. Eidenschenk, S. Rutz, O. Liesenfeld, et al., Role of IL-22 in microbial host defense, Curr. Top. Microbiol. Immunol. 380 (2014) 213–236.
[2] K. Eyerich, V. Dimartino, A. Cavani, IL-17 and IL-22 in immunity: driving protection and pathology, Eur. J. Immunol. 47 (2017) 607–614.
[3] H. Fujita, The role of IL-22 and Th22 cells in human skin diseases, J. Dermatol. Sci. 72 (2013) 3–8.
[4] K. Boniface, F.X. Bernard, M. Garcia, et al., IL-22 inhibits epidermal differentiation and induces proinflammatory gene expression and migration of human keratinocytes, J. Immunol. 174 (2005) 3695–3702.
[5] K. Wolk, E. Witte, E. Wallace, et al., IL-22 regulates the expression of genes responsible for antimicrobial defense, cellular differentiation, and mobility in keratinocytes: a potential role in psoriasis, Eur. J. Immunol. 36 (2006) 1309–1323.
[6] S.M. Sa, P.A. Valdez, J. Wu, et al., The effects of IL-20 subfamily cytokines on reconstituted human epidermis suggest potential roles in cutaneous innate defense and pathogenic adaptive immunity in psoriasis, J. Immunol. 178 (2007) 3229–3240.
[7] K. Wolk, S. Kunz, E. Witte, et al., IL-22 increases the innate immunity of tissues, Immunity 21 (2004) 241–254.
[8] S. Buta, H. Edamatsu, M. Li, et al., Crucial role of phospholipase C epsilon in skin inflammation induced by tumor-promoting phorbol ester, Canc. Res. 68 (2008) 64–72.
[9] L. Hu, H. Edamatsu, N. Takenaka, et al., Crucial role of phospholipase C epsilon in induction of local skin inflammatory reactions in the elicitation stage of allergic contact hypersensitivity, J. Immunol. 184 (2010) 993–1002.
[10] N. Takenaka, H. Edamatsu, N. Suzuki, et al., Overexpression of phospholipase C epsilon in keratinocytes upregulates cytokine expression and causes dermaitis with acanthosis and T-cell infiltration, Eur. J. Immunol. 41 (2011) 202–213.
[11] Y. Bai, H. Edamatsu, S. Maeda, et al., Crucial role of phospholipase C epsilon in chemical carcinogen-induced skin tumor development, Canc. Res. 64 (2004) 8808–8810.
[12] M. Tanado, H. Edamatsu, S. Minamisawa, et al., Congenital semilunar valve volegenesis defect in mice deficient in phospholipase C epsilon, Mol. Cell Biol. 25 (2005) 2191–2199.
[13] X. Zhu, Y. Sun, X. Mu, et al., Phospholipase C epsilon deficiency delays the early stage of cutaneous wound healing and attenuates scar formation in mice, Biochem. Biophys. Res. Commun. 484 (2017) 144–151.
[14] M. Oka, H. Edamatsu, M. Kunisada, et al., Phospholipase C epsilon has a crucial role in ultraviolet B-induced neutrophil-associated skin inflammation by regulating the expression of CXCL1/KC, Laboratory investigation, J. Tech. Methods Pathol. 91 (2011) 711–718.
[15] W. Ouyang, S. Rutz, N.K. Crellin, et al., Regulation and functions of the IL-10 family of cytokines in inflammation and disease, Annu. Rev. Immunol. 29 (2011) 71–109.
[16] P.M. Elias, K.R. Feingold, Coordinate regulation of epidermal differentiation and barrier homeostasis, Skin Pharmacol. Appl. Skin Physiol. 14 (Suppl 1) (2001) 28–34.
[17] H.R. Smith, M. Rowson, D.A. Baskett, et al., Intra-individual variation of irritant threshold and relationship to transepidermal water loss measurement of skin irritation, Contact Dermatitis 51 (2004) 26–29.
[18] R. Darlenski, J. Kazandjieva, N. Tsankov, et al., Acute irritant threshold correlates with barrier function, skin hydration and contact hypersensitivity in atopic dermatitis and rosacea, Exp. Dermatol. 22 (2013) 752–753.
[19] L. Ye, C. Lv, G. Man, et al., Abnormal epidermal barrier recovery in uninvolved skin supports the notion of an epidermal pathogenesis of psoriasis, J. Invest. Dermatol. 134 (2014) 2843–2846.
[20] Y.H. Lo, K. Torii, C. Saito, et al., Serum IL-22 correlates with psoriatic severity and serum IL-6 correlates with susceptibility to phototherapy, J. Dermatol. Sci. 58 (2010) 225–227.
[21] K. Wolk, H.S. Haugen, W. Xu, et al., IL-22 and IL-20 are key mediators of the epidermal alterations in psoriasis while IL-17 and IFN-gamma are not, J. Mol. Med.-Jmm 87 (2009) 523–536.
[22] H.L. Ma, S. Liang, J. Li, et al., IL-22 is required for Th17 cell-mediated pathology in a mouse model of psoriasis-like skin inflammation, J. Clin. Invest. 118 (2008) 597–607.
[23] A.B. Van Belle, M. de Herstch, M.M. Lemaire, et al., IL-22 is required for imiquimod-induced psoriasis formation in mice, J. Immunol. 188 (2012) 462–469.