If the link missed: Could inflammatory skin disorders with barrier dysfunction increase the risk of COVID-19?

Xu Qiannan, MD*; Chen Lihong, MD, PhD*; Zhang Li, MD*; Li Xia, MD, PhD†; Zheng Jie, MD, PhD†.

Department of Dermatology, Ruijin Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai 200025, P.R. China

*These authors contributed equally to this work.

†Corresponding author. Email: jie-zheng2001@126.com (Z.J.); drlixia@126.com (L.X.);

ABSTRACT

Since the end of 2019, COVID-19 pandemic caused by the SARS-CoV-2 emerged globally. The angiotensin-converting enzyme 2 (ACE2) on the cell surface is crucial for SARS-COV-2 entering into the cells. The expression of ACE2 in skin suggested that skin might be a way of transmitting SARS-CoV-2. We found the elevated ACE2 level of patients with psoriasis but downregulated after IL-17 antibody treatment. Further results showed that ACE2 expression increased either in psoriasis or in atopic dermatitis, which were typical inflammatory skin disorders with barrier dysfunction. And elevated ACE2 level was also detected in mouse models of dermatitis induced by imiquimod, calcipotriol, repeated tape-stripping or 1-Fluoro-2,4-dinitrobenzene (DNFB) respectively. Moreover, alleviation of cutaneous inflammation with skin recovery moisture also lowered expression of ACE2 in mouse models with barrier deteriorated inflammatory skin disorders. Furthermore, inflammatory skin disorders with barrier dysfunction increased the penetration of topical FITC conjugated spike protein into the skin. Conversely, improvement in skin permeability barrier could prevent this penetration. Thus, indicating the special link between inflammatory skin disorders with skin barrier dysfunction and increasing risk of COVID-19.

Since the end of 2019, a new corona virus emerged and widely spread throughout the world (1). This virus was further recognized as SARS-CoV-2 (2). By using its spike protein (3) SARS-CoV-2 could bind angiotensin-converting enzyme 2 (ACE2) on alveolar epithelial type II cells

(4) in the lung and cause severe lung injury, multi-organ failure, and death (5). Respiratory tract was recognized as the major way of transmission (6). Higher expression of ACE2 was thought to be associated with elevated susceptibility to SARS-CoV-2 (7). However, wearing a mask only reduce the risk of SARS-CoV-2 through respiratory tract (8). The possibility of SARS-CoV-2 infection through other tissue, especially in those with expression of ACE2, could not be excluded (9). Thus, either downregulation of ACE2 expression or prevention ACE2 expressing cells from contacting of SARS-CoV-2 could be a solution for preventing the disease (10). One of the well observed potential target organ of SARS-CoV-2 is gut (9, 11) because it held a high ACE2 expression and several symptoms related to digestive systems as diarrhea, vomiting, and abdominal pain were observed in COVID-19 patients (12). Similar to gut, skin could also express ACE2 (13-15) and cases were reported that patients with COVID-19 could have vascular skin symptoms (16), varicella-like exanthem (17), and pernio-like skin lesions (18) which were all symptoms occurred on skin. Besides that, SARS-CoV-2 immunohistochemistry was positive in endothelial cells and epithelial cells of eccrine glands in skin (19). Thus, it indicated that skin as a first line defensing barrier of human body which constantly exposure to various pathogens (20) could be another potential target organ of SARS-CoV-2.

When an organ become a potential organ of transmitting SARS-CoV-2 there were to be concern that nowadays widely used biological agents which treating diseases of that organ could elevate the susceptibility to SARS-CoV-2 (21). Because using biologics means more vulnerable to various pathogens (22). These concerns were in dermatologists as well. Psoriasis as an immune-mediated, genetic related disease manifesting in the skin with high prevalence, chronicity, disfiguration, disability, and associated comorbidity (23), biologics played an important role in treating it. However, with the pandemic of COVID-19, whether using biologics could elevated the susceptibility to SARS-CoV-2 remained unknown (24-26). Using biologics treat skin disease in COVID-19 pandemic has its controversial portrait. Although cases reported severe infection occurred after biologics treatment (27), biologics could somehow reduce the possibility of transmitting pathogens through skin. After biologics treatment the inflammation were gone and deteriorated skin barrier function was back to normal. The widened physical gap between keratinocytes of dysfunctional skin barrier returned to the normal skin barrier with tightly conjunct keratinocytes (28). This contribute to the defensing of various pathogens from skin (29), herein during the COVID-19 pandemic could be SARS-CoV-2.

To evaluate the influence of biologics on the inflammatory skin with barrier dysfunction, we randomly select 5 psoriasis patients who were under the treatment of IL-17 antibody (Taltz, Eli Lilly and Company). The lesion skin of patients was obtained on week 0 and week 8 after first use of the IL-17 antibody. ACE2 mRNA expression detection was prepared as previously described (30). The skin ACE2 mRNA expression of patients underwent using IL-17 antibody for 8 weeks is downregulated compared with the ACE2 expression in the skin on week 0 when the IL-17 antibody treatment started (Fig. 1A). To confirm the result, we randomly select 3 patients under the IL-17 antibody treatment and skin of patients were biopsied on week 0 and week 8 after first use of the IL-17 antibody. The tissue was prepared for

immunofluorescence stain as previously described (31). The immunofluorescence stain revealed the fluorescence intensity of ACE2 downregulated in the patient skin underwent IL-17 antibody treatment on week 8 compared with the patient skin before the IL-17 antibody (Fig. 1, B to D). We also tested CD209, a receptor is associated with ACE2, which could enhance ACE2 mediated virus binding and cellular entry (32). However, the data showed no significant difference between 0w and 8w. Data above showed biologics might contribute to the defensing of SARS-CoV-2 in inflammatory skin disorders with barrier dysfunction through modulating skin barrier function and downregulating ACE2 expression.

Higher ACE2 expression indicated higher susceptibility to SARS-COV-2 which implied that skin disorder with barrier dysfunction might increase the risk of COVID-19. Since the treatment of psoriasis, a typical skin inflammatory disorder with barrier dysfunction could downregulate the expression of ACE2 in skin, could inflammation with barrier dysfunction been the mediator of ACE2 expression and then affect the possible risk of SARS-COV-2 transmission through skin?

To evaluate whether skin inflammatory disorder with barrier dysfunction have elevated ACE2 level which might enhance the possibility of transmitting of SARS-CoV-2 in skin, we randomly select 5 samples of psoriasis, 5 samples of atopic dermatitis which were all with skin barrier dysfunction and 3 samples without barrier dysfunction (All obtained from patients with nevus). Immunohistochemistry stain was prepared to all the samples. The ACE2 expressed mainly in the stratum basale and dermal (Fig. 2, A to C). In the IHC stain of atopic dermatitis ACE2 positive stain could observed on the higher layer when compared with psoriasis and skin without barrier dysfunction. While in dermal the percentage of ACE2 positive cells in psoriasis and atopic dermatitis IHC stain were recognized mostly as infiltrated inflammatory cells. The ACE2 expression in the IHC stain was measured by Image J IHC profiler plugin. ACE2 expression of psoriasis and atopic dermatitis is significantly elevated as compared with the skin without barrier dysfunction (Fig. 2D). These results showed skin ACE2 level of inflammatory skin disorders with barrier dysfunction were upregulated compared with the skin without barrier dysfunction.

Then does other inflammatory skin disorders with barrier dysfunction share the same high ACE2 expression portrait? We further established four different mouse models, including psoriasis, atopic dermatitis mouse models induced by imiquimod or calcipotriol respectively, acute skin inflammatory skin disorder induced repeated tape-stripping (33) as well as 1-Fluoro-2,4-dinitrobenzene (DNFB). All four models were recognized as mouse models of inflammatory skin disorder with deteriorated barrier function. Paired control without skin inflammatory skin disorder and barrier dysfunction was established in the same length of time (Imiquimod control group was applied with vaseline while calcipotriol and DNFB group control was applied with absolute ethanol, mechanism skin barrier dysfunction control was established by eliminate mechanism treatment). The ACE2 mRNA expression of imiquimod group, calcipotriol group, mechanical barrier disruption group and DNFB group were all significant elevated compared with paired group control (Fig. 3A). Furthermore, the deviation of ACE2 mRNA expression in imiquimod induced psoriasis mouse model and calcipotriol

induced atopic dermatitis mouse model compared with control group were significantly higher than the deviation of ACE2 mRNA expression in mouse models with barrier dysfunction however not mimicking the most recognized typical skin inflammatory disorder (Fig. 3B). Besides that, when applied with skin recovery moisture (Linoleic acid-ceramide moisturizer recognized as sufficient in prevent psoriasis and atopic dermatitis relapse trough skin barrier recovery and alleviate inflammation) (34,35) the mRNA expression of ACE2 in the imiquimod treated group and calcipotriol treated group was downregulated as compared with the skin recovery moisture untreated group (Fig. 3A).

The attachment of spike protein (S protein) with ACE2 is necessary for the transmission of SARS-COV-2. Then could the skin inflammatory disorder with barrier dysfunction affect the S protein entering skin? The influence of skin inflammation and barrier function to the binding of ACE2 and SARS-COV-2 was evaluated. Mouse model of skin inflammatory disorder with barrier dysfunction induced by imiquimod was established and group control was established as applied with vehicle cream (Vaseline Lanette cream, Fargon). Then FITC conjugated S protein was applied to the skin. After that the treated skin was prepared for an immunofluorescence stain. The FITC conjugated S protein was detected depositing in the dermal of mouse model with inflammatory skin disorder and deteriorated skin barrier function (Fig. 4B) while there was none immunofluorescence intensity detected in the mouse model with normal barrier function and without skin inflammatory disorder (Fig. 4A). The FACS scan result of the skin FITC conjugated S protein marked cells were significantly higher in the skin barrier dysfunction group than the control group while by applying the skin barrier recovery moisture the quantity of FITC conjugated S protein permeated the skin barrier significantly reduced (Fig. 4, C to F).

With the pandemic of COVID-19, the method to prevent SARS-COV-2 from spreading become more and more urgent. Reveal the possible way of SARS-COV-2 transmitting may encourage new method reduce the COVID-19 spreading. The expression of ACE2 in skin gives the possibility that skin might be another link to the COVID-19 transmission. Our work not only support the data recently published that ACE2 expressed in skin and in atopic dermatitis, a typical inflammatory skin disorder with barrier dysfunction, ACE2 gene level was elevated (36), but also added that besides atopic dermatitis, another inflammatory skin disorder with barrier dysfunction held elevated ACE2 level in lesion. There is one thing should be noted that the expression of ACE2 is not on the surface of skin, however, it mainly expressed in the dermal and the cells between dermal and epidermal. Deteriorated skin barrier gives various microbes the opportunity to get through the baseline of epidermal (29) and so does SARS-COV-2 especially in the inflammation state. In our study we recognized that besides the already known physical dysfunction of the skin barrier which may lead the COVID-19 S protein binding the ACE2 expressing cell in the stratum basale and dermal, upregulation of ACE2 in the skin with barrier dysfunction were also detected in our study. These dermal ACE2 positive cells held an infiltrating inflammatory cell look. And there's a possibility that these cells could be macrophage (37) or dendritic cells (38) which indicated that inflammatory atmosphere along with the deteriorated skin barrier could be the front door SARS-COV-2 knocked on. The elevated ACE2 level in the skin inflammatory disorder

with deteriorated barrier provide the suitable environment for the transmission of SARS-COV-2. We also divided the skin inflammatory disorder with deteriorated barrier models into different groups and the models related more to inflammation (39-41) seemed to have higher ACE2 expression. As in our previous study the relieve of psoriasis was followed by the recovery of skin barrier (34), our data now revealed the skin ACE2 expression downregulated along with the alleviation of psoriatic skin barrier dysfunction due to IL-17 antibody treatment which could be a support that IL-17 antibody treatment in psoriasis might affect the COVID-19 transmission in a preventive way. The expression of CD209 showed no difference before and after the treatment, i which should be further investigated. Besides that, skin barrier recovery moisture which could also alleviated the inflammation as well as protect the skin barrier from dysfunction showed potential in reducing the risk of COVID-19 infection transmission. Deteriorated barrier function makes the virus easier to enter the skin, while the increased expression of ACE2 makes the virus easier to enter the cell. Inflammatory skin disorders (including atopic dermatitis and psoriasis) are accompanied by deteriorated barrier function and increased ACE2 expression, therefore, it is possible to increase the risk of COVID-19. (Fig. 5) Our work not only provided a new insight into the COVID-19 transmission way and provoked further studies in the P4 lab but also a support for those who were fighting with stubborn protective clothing as well as an alert to those whose skin exposure to the virus without a proper protection.

REFERENCE AND NOTES:

1. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, Ren R, Leung KSM, Lau EHY, Wong JY, Xing X, Xiang N, Wu Y, Li C, Chen Q, Li D, Liu T, Zhao J, Liu M, Tu W, Chen C, Jin L, Yang R, Wang Q, Zhou S, Wang R, Liu H, Luo Y, Liu Y, Shao G, Li H, Tao Z, Yang Y, Deng Z, Liu B, Ma Z, Zhang Y, Shi G, Lam TTY, Wu JT, Gao GF, Cowling BJ, Yang B, Leung GM, Feng Z. Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia. N Engl J Med. 2020 Mar 26;382(13):1199-1207. doi: 10.1056/NEJMoa2001316. Epub 2020 Jan 29. PMID: 31995857; PMCID: PMC7121484.
2. World Health Organization. Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-19) 16-24 February 2020 [Internet]. Geneva: World Health Organization; 2020 Available from: https://www.who.int/docs/default-source/coronaviruse/who-china-joint-mission-on-covid-19-final-report.pdf
3. Yan R, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. Science. 2020 Mar 27;367(6485):1444-1448. doi: 10.1126/science.abb2762. Epub 2020 Mar 4. PMID: 32132184; PMCID: PMC7164635.
4. Ziegler CGK, Allon SJ, Nyquist SK, Mbano IM, Miao VN, Tzouanas CN, Cao Y, Yousif AS, Bals J, Hauser BM, Feldman J, Muus C, Wadsworth MH 2nd, Kazer SW, Hughes TK, Doran B, Gatter GJ, Vukovic M, Taliaferro F, Mead BE, Guo Z, Wang JP, Gras D, Plaisant M, Ansari M, Angelidis I, Adler H, Sucre JMS, Taylor CJ, Lin B, Waghray A, Mitsialis V, Dwyer DF, Buchheit KM, Boyce JA, Barrett NA, Laidlaw TM, Carroll SL, Colonna L, Tkachev V, Peterson CW, Yu A, Zheng HB, Gideon HP, Winchell CG, Lin PL, Bingle CD, Snapper SB, Kropski JA, Theis FJ, Schiller HB, Zaragosi LE, Barbry P, Leslie A, Kiem HP, Flynn JL, Fortune SM, Berger B, Finberg RW, Kean LS, Garber M, Schmidt AG, Lingwood D, Shalek AK, Ordovas-Montanes J; HCA Lung Biological Network. Electronic address: lung-

network@humancellatlas.org; HCA Lung Biological Network. SARS-CoV-2 Receptor ACE2 Is an Interferon-Stimulated Gene in Human Airway Epithelial Cells and Is Detected in Specific Cell Subsets across Tissues. Cell. 2020 May 28;181(5):1016-1035.e19. doi: 10.1016/j.cell.2020.04.035. Epub 2020 Apr 27. PMID: 32413319; PMCID: PMC7252096.

5. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J, Cao B. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020 Feb 15;395(10223):497-506. doi: 10.1016/S0140-6736(20)30183-5. Epub 2020 Jan 24. Erratum in: Lancet. 2020 Jan 30;: PMID: 31986264; PMCID: PMC7159299.
6. Chan JF, Yuan S, Kok KH, To KK, Chu H, Yang J, Xing F, Liu J, Yip CC, Poon RW, Tsoi HW, Lo SK, Chan KH, Poon VK, Chan WM, Ip JD, Cai JP, Cheng VC, Chen H, Hui CK, Yuen KY. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet. 2020 Feb 15;395(10223):514-523. doi: 10.1016/S0140-6736(20)30154-9. Epub 2020 Jan 24. PMID: 31986261; PMCID: PMC7159286.
7. Smith JC, Sausville EL, Girish V, Yuan ML, Vasudevan A, John KM, Sheltzer JM. Cigarette Smoke Exposure and Inflammatory Signaling Increase the Expression of the SARS-CoV-2 Receptor ACE2 in the Respiratory Tract. Dev Cell. 2020 Jun 8;53(5):514-529.e3. doi: 10.1016/j.devcel.2020.05.012. Epub 2020 May 16. PMID: 32425701; PMCID: PMC7229915.
8. Javid B, Weekes MP, Matheson NJ. Covid-19: should the public wear face masks? BMJ. 2020 Apr 9;369:m1442. doi: 10.1136/bmj.m1442. PMID: 32273278.
9. Lamers MM, Beumer J, van der Vaart J, Knoops K, Puschhof J, Breugem TI, Ravelli RBG, Paul van Schayck J, Mykytyn AZ, Duimel HQ, van Donselaar E, Riesebosch S, Kuijpers HJH, Schippers D, van de Wetering WJ, de Graaf M, Koopmans M, Cuppen E, Peters PJ, Haagmans BL, Clevers H. SARS-CoV-2 productively infects human gut enterocytes. Science. 2020 May 1:eabc1669. doi: 10.1126/science.abc1669. Epub ahead of print. PMID: 32358202; PMCID: PMC7199907.
10. Wu Y, Wang F, Shen C, Peng W, Li D, Zhao C, Li Z, Li S, Bi Y, Yang Y, Gong Y, Xiao H, Fan Z, Tan S, Wu G, Tan W, Lu X, Fan C, Wang Q, Liu Y, Zhang C, Qi J, Gao GF, Gao F, Liu L. A noncompeting pair of human neutralizing antibodies block COVID-19 virus binding to its receptor ACE2. Science. 2020 Jun 12;368(6496):1274-1278. doi: 10.1126/science.abc2241. Epub 2020 May 13. PMID: 32404477; PMCID: PMC7223722.
11. Lamers MM, Beumer J, van der Vaart J, Knoops K, Puschhof J, Breugem TI, Ravelli RBG, Paul van Schayck J, Mykytyn AZ, Duimel HQ, van Donselaar E, Riesebosch S, Kuijpers HJH, Schippers D, van de Wetering WJ, de Graaf M, Koopmans M, Cuppen E, Peters PJ, Haagmans BL, Clevers H. SARS-CoV-2 productively infects human gut enterocytes. Science. 2020 May 1:eabc1669. doi: 10.1126/science.abc1669. Epub ahead of print. PMID: 32358202; PMCID: PMC7199907.
12. Wong SH, Lui RN, Sung JJ. Covid-19 and the digestive system. J Gastroenterol Hepatol. 2020 May;35(5):744-748. doi: 10.1111/jgh.15047. Epub 2020 Apr 19. PMID: 32215956.
13. Grzegrzolka J, Swiatko K, Pula B, Zamirska A, Olbromski M, Bieniek A, Szepietowski J, Rys J, Dziegiel P, Podhorska-Okolow M. ACE and ACE2 expression in normal and

malignant skin lesions. Folia Histochem Cytobiol. 2013;51(3):232-8. doi: 10.5603/FHC.2013.0033. PMID: 24203630.

14. Hamming I, Timens W, Bulthuis ML, Lely AT, Navis G, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathol. 2004 Jun;203(2):631-7. doi: 10.1002/path.1570. PMID: 15141377; PMCID: PMC7167720.
15. Xue X, Mi Z, Wang Z, Pang Z, Liu H, Zhang F. High expression of ACE2 on the keratinocytes reveals skin as a potential target for SARS-CoV-2. J Invest Dermatol. 2020 May 23:S0022-202X(20)31602-X. doi: 10.1016/j.jid.2020.05.087. Epub ahead of print. PMID: 32454066; PMCID: PMC7245327.
16. Bouaziz JD, Duong T, Jachiet M, Velter C, Lestang P, Cassius C, Arsouze A, Domergue Than Trong E, Bagot M, Begon E, Sulimovic L, Rybojad M. Vascular skin symptoms in COVID-19: a french observational study. J Eur Acad Dermatol Venereol. 2020 Apr 27:10.1111/jdv.16544. doi: 10.1111/jdv.16544. Epub ahead of print. PMID: 32339344; PMCID: PMC7267662.
17. Marzano AV, Genovese G, Fabbrocini G, Pigatto P, Monfrecola G, Piraccini BM, Veraldi S, Rubegni P, Cusini M, Caputo V, Rongioletti F, Berti E, Calzavara-Pinton P. Varicella-like exanthem as a specific COVID-19-associated skin manifestation: Multicenter case series of 22 patients. J Am Acad Dermatol. 2020 Apr 16;83(1):280–5. doi: 10.1016/j.jaad.2020.04.044. Epub ahead of print. PMID: 32305439; PMCID: PMC7161488.
18. Freeman EE, McMahon DE, Lipoff JB, Rosenbach M, Kovarik C, Takeshita J, French LE, Thiers BH, Hruza GJ, Fox LP; American Academy of Dermatology Ad Hoc Task Force on Covid-19. Pernio-like skin lesions associated with COVID-19: a case series of 318 patients from 8 countries. J Am Acad Dermatol. 2020 May 29:S0190-9622(20)30984-1. doi: 10.1016/j.jaad.2020.05.109. Epub ahead of print. PMID: 32479979; PMCID: PMC7260509.
19. Colmenero I, Santonja C, Alonso-Riaño M, Noguera-Morel L, Hernández-Martín A, Andina D, Wiesner T, Rodríguez-Peralto JL, Requena L, Torrelo A. SARS-CoV-2 endothelial infection causes COVID-19 chilblains: histopathological, immunohistochemical and ultraestructural study of 7 paediatric cases. Br J Dermatol. 2020 Jun 20. doi: 10.1111/bjd.19327. Epub ahead of print. PMID: 32562567.
20. Kupper TS, Fuhlbrigge RC. Immune surveillance in the skin: mechanisms and clinical consequences. Nat Rev Immunol. 2004 Mar;4(3):211-22. doi: 10.1038/nri1310. PMID: 15039758; PMCID: PMC7097017.
21. Potdar AA, Dube S, Naito T, Botwin G, Haritunians T, Li D, Yang S, Bilsborough J, Denson LA, Daly M, Targan SR, Fleshner P, Braun J, Kugathasan S, Stappenbeck TS, McGovern DPB. Reduced expression of COVID-19 host receptor, *ACE2* is associated with small bowel inflammation, more severe disease, and response to anti-TNF therapy in Crohn's disease. medRxiv [Preprint]. 2020 Apr 23:2020.04.19.20070995. doi: 10.1101/2020.04.19.20070995. PMID: 32511625; PMCID: PMC7276052.
22. Click B, Regueiro M. Managing Risks with Biologics. Curr Gastroenterol Rep. 2019 Jan 11;21(2):1. doi: 10.1007/s11894-019-0669-6. PMID: 30635807.
23. Boehncke WH, Schön MP. Psoriasis. Lancet. 2015 Sep 5;386(9997):983-94. doi:

10.1016/S0140-6736(14)61909-7. Epub 2015 May 27. PMID: 26025581.
24. Amerio P, Prignano F, Giuliani F, Gualdi G. COVID-19 and psoriasis: Should we fear for patients treated with biologics? [published online ahead of print, 2020 Apr 20]. *Dermatol Ther*. 2020;e13434. doi:10.1111/dth.13434
25. Lebwohl M, Rivera-Oyola R, Murrell DF. Should biologics for psoriasis be interrupted in the era of COVID-19? J Am Acad Dermatol. 2020 May;82(5):1217-1218. doi: 10.1016/j.jaad.2020.03.031. Epub 2020 Mar 19. PMID: 32199889; PMCID: PMC7156810.
26. Bardazzi F, Loi C, Sacchelli L, Di Altobrando A. Biologic therapy for psoriasis during the covid-19 outbreak is not a choice. J Dermatolog Treat. 2020 Jun;31(4):320-321. doi: 10.1080/09546634.2020.1749545. Epub 2020 Apr 6. PMID: 32248724.
27. Oussedik E, Patel NU, Cash DR, Gupta AS, Feldman SR. Severe and acute complications of biologics in psoriasis. G Ital Dermatol Venereol. 2017 Dec;152(6):586-596. doi: 10.23736/S0392-0488.17.05750-9. Epub 2017 Sep 12. PMID: 28895664.
28. Dainichi T, Kitoh A, Otsuka A, Nakajima S, Nomura T, Kaplan DH, Kabashima K. The epithelial immune microenvironment (EIME) in atopic dermatitis and psoriasis. Nat Immunol. 2018 Dec;19(12):1286-1298. doi: 10.1038/s41590-018-0256-2. Epub 2018 Nov 16. PMID: 30446754.
29. Byrd AL, Belkaid Y, Segre JA. The human skin microbiome. Nat Rev Microbiol. 2018 Mar;16(3):143-155. doi: 10.1038/nrmicro.2017.157. Epub 2018 Jan 15. PMID: 29332945.
30. Wang L, Li K, Xu Q, Chen X, Xue F, Shi R, Zheng J. Potential synergy between SNP and CpG-A or IL-1β in regulating transcriptional activity of IL-20 promoter. J Invest Dermatol. 2014 Feb;134(2):389-395. doi: 10.1038/jid.2013.314. Epub 2013 Jul 26. PMID: 23892591.
31. Li Q, Ke F, Zhang W, Shen X, Xu Q, Wang H, Yu XZ, Leng Q, Wang H. Plasmin plays an essential role in amplification of psoriasiform skin inflammation in mice. PLoS One. 2011 Feb 2;6(2):e16483. doi: 10.1371/journal.pone.0016483. PMID: 21311769; PMCID: PMC3032787.
32. Brufsky A, Lotze MT. DC/L-SIGNs of Hope in the COVID-19 Pandemic. J Med Virol. 2020 May 6:10.1002/jmv.25980. doi: 10.1002/jmv.25980. Epub ahead of print. PMID: 32374430; PMCID: PMC7267543.
33. Wang H, Baran U, Wang RK. In vivo blood flow imaging of inflammatory human skin induced by tape stripping using optical microangiography. J Biophotonics. 2015 Mar;8(3):265-72. doi: 10.1002/jbio.201400012. Epub 2014 Mar 21. PMID: 24659511; PMCID: PMC4308563.
34. Liu M, Li X, Chen XY, Xue F, Zheng J. Topical application of a linoleic acid-ceramide containing moisturizer exhibit therapeutic and preventive benefits for psoriasis vulgaris: a randomized controlled trial. Dermatol Ther. 2015;28(6):373-382. doi:10.1111/dth.12259
35. Yang Q, Liu M, Li X, Zheng J. The benefit of a ceramide-linoleic acid-containing moisturizer as an adjunctive therapy for a set of xerotic dermatoses. Dermatol Ther. 2019 Jul;32(4):e13017. doi: 10.1111/dth.13017. Epub 2019 Jul 28. PMID: 31276265.
36. Radzikowska U, Ding M, Tan G, Zhakparov D, Peng Y, Wawrzyniak P, Wang M, Li S, Morita H, Altunbulakli C, Reiger M, Neumann AU, Lunjani N, Traidl-Hoffmann C, Nadeau K, O'Mahony L, Akdis CA, Sokolowska M. Distribution of ACE2, CD147, CD26 and other

SARS-CoV-2 associated molecules in tissues and immune cells in health and in asthma, COPD, obesity, hypertension, and COVID-19 risk factors. Allergy. 2020 Jun 4. doi: 10.1111/all.14429. Epub ahead of print. PMID: 32496587.

37. Sluimer JC, Gasc JM, Hamming I, van Goor H, Michaud A, van den Akker LH, Jütten B, Cleutjens J, Bijnens AP, Corvol P, Daemen MJ, Heeneman S. Angiotensin-converting enzyme 2 (ACE2) expression and activity in human carotid atherosclerotic lesions. J Pathol. 2008 Jul;215(3):273-9. doi: 10.1002/path.2357. PMID: 18498093.
38. Yang J, Li H, Hu S, Zhou Y. ACE2 correlated with immune infiltration serves as a prognostic biomarker in endometrial carcinoma and renal papillary cell carcinoma: implication for COVID-19. Aging (Albany NY). 2020 Apr 27;12(8):6518-6535. doi: 10.18632/aging.103100. Epub 2020 Apr 27. PMID: 32339157; PMCID: PMC7202533.
39. Bäsler K, Brandner JM. Tight junctions in skin inflammation. Pflugers Arch. 2017 Jan;469(1):3-14. doi: 10.1007/s00424-016-1903-9. Epub 2016 Nov 16. PMID: 27853878.
40. Jensen JM, Proksch E. The skin's barrier. G Ital Dermatol Venereol. 2009 Dec;144(6):689-700. PMID: 19907407.
41. Egawa G, Kabashima K. Multifactorial skin barrier deficiency and atopic dermatitis: Essential topics to prevent the atopic march. J Allergy Clin Immunol. 2016 Aug;138(2):350-358.e1. doi: 10.1016/j.jaci.2016.06.002. Epub 2016 Jun 22. PMID: 27497277.

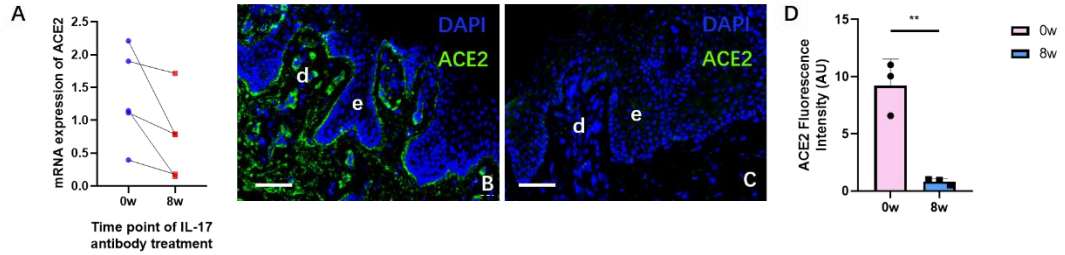

Fig 1. **The expression of ACE2 in the lesion before and after the IL-17 antibody treatment.** (A) The skin ACE2 mRNA expression in the lesion before (0w) and 8 weeks after (8w) the IL-17 antibody treatment. (B and C) Immunofluorescence stain of ACE2 in the patient skin that underwent IL-17 antibody treatment before (B) and 8 weeks after (C). Bar indicated 10μm. (D) The comparison of ACE2 immunofluorescence stain in the patient skin before and 8 weeks after the IL-17 antibody treatment. Bars indicate mean ± SD of experiment groups. ** P < 0.05, Student's t test.

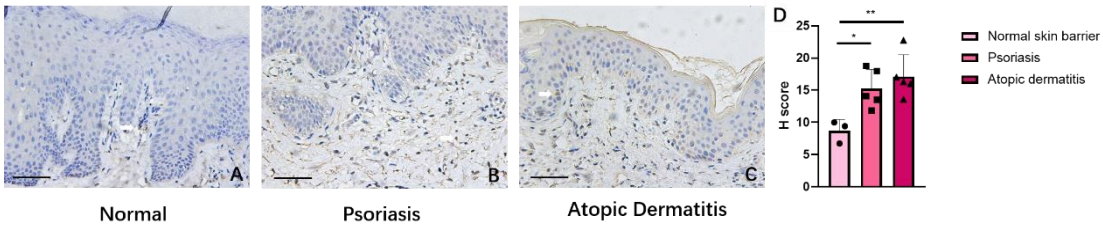

Fig 2. **The IHC stain of ACE2 in the skin tissue with different skin conditions.** (A-C) The ACE2 expression of skin with normal skin barrier function (A), with psoriasis (B) and with atopic dermatitis (C). Bar indicated 10 μm. Arrow indicated positive stain. (D) The comparison of ACE2 expression in the skin with normal barrier function, lesion of psoriasis patient and lesion of patient with atopic dermatitis. Bars indicate mean±SD of experiment groups. ** P < 0.05. Student's t test.

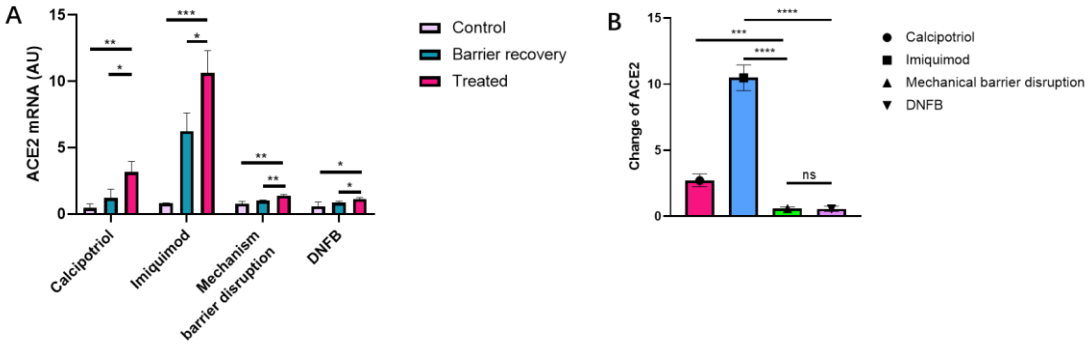

Fig 3. **The ACE2 mRNA expression of different inflammatory disorders with skin barrier dysfunction mouse models.** (A) Comparison of ACE2 mRNA expression of different inflammatory disorders with skin barrier dysfunction mouse models. (B) Comparison of ACE2 mRNA expression change of model establish before and after in different mouse model groups. Bars indicate mean±SD of experiment groups. ** P < 0.05, *** P < 0.01 and **** P < 0.001 P < 0.01. Student's t test.

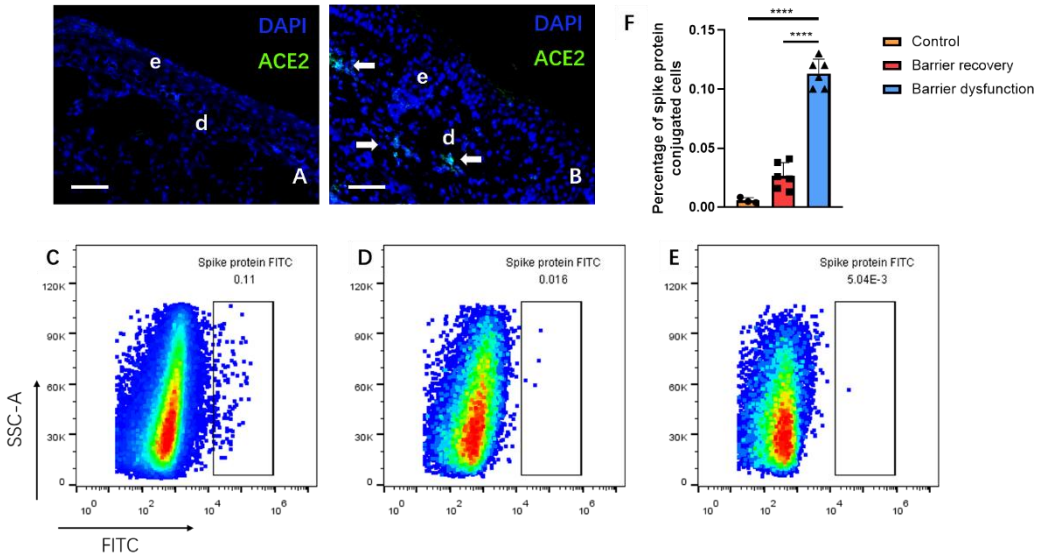

Fig 4. **Deposition of FITC conjugated spike protein of mouse models with different skin condition.** (A and B) Immunofluorescence stain of mouse without (A) or with (B) skin barrier dysfunction after applied with FITC conjugated spike protein. Bar indicated 10μm. Arrow indicated the deposition of spike protein. (C-E) Quantity of spike protein conjugated cell in the skin with barrier dysfunction and applied with FITC marked spike protein without (C) or with (D) barrier recovery moisture and the skin without barrier dysfunction (E) applied with FITC marked spike protein detected by FACS. (F) The comparison of the percentage of spike protein conjugated cells in the skin of different groups with skin barrier function. Bars indicate mean±SD of experiment groups. ** $P < 0.05$, *** $P < 0.01$ and **** $P < 0.001$ $P < 0.01$. Student's t test.

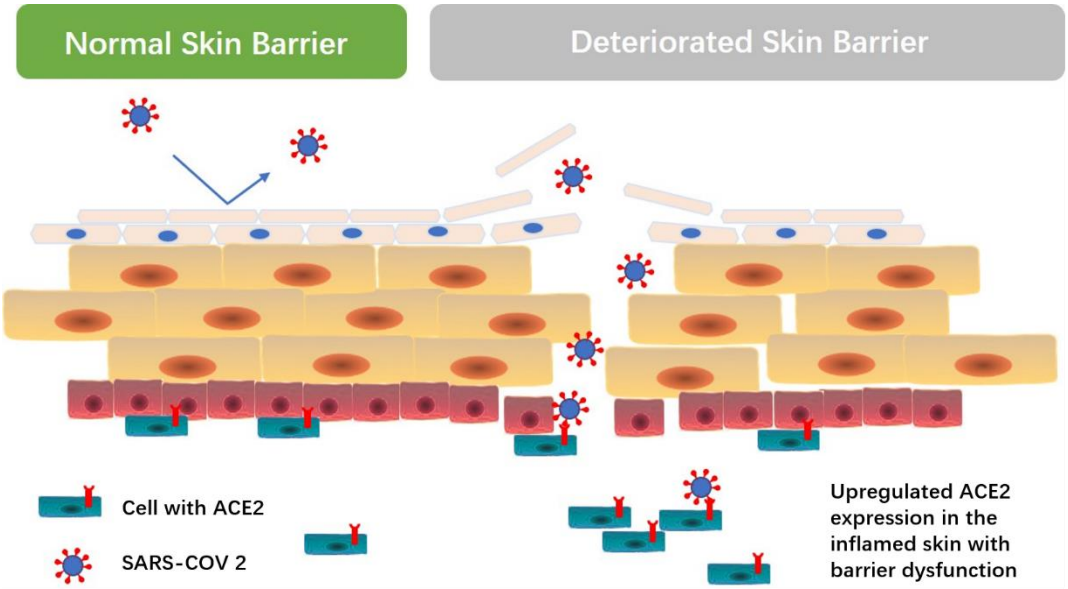

Fig 5. **Possible mechanism SARS-COV 2 transmission through deteriorated skin barrier.**

Materials and Methods

Human tissue specimens

All procedures and use of (anonymized) tissue were performed according to recent national ethical guidelines. Human skin were obtained from patients undergoing biopsy procedures for diagnostic purposes or surgery for various reasons.

Immunohistochemistry

Tissue samples were fixed in 10% buffered formalin, dehydrated, and embedded in paraffin. Paraffin sections, 6 µm thick, were stained with haematoxylin and eosin (H&E) to verify the diagnosis. ACE2 and Ki-67 IHC staining was performed on 4-µm-thick paraffin sections fixed on Superfrost Plus slides (Menzel Gläser, Braunschweig, Germany). Deparaffinization and antigen retrieval were performed in Target Retrieval Solution with pH 9 (ACE2) or pH 6 (Ki-67) at 97°C for 20 min using the PT Link platform (Dako, Glostrup, Denmark). Subsequently, the sections were washed in TBS (tris-buffered saline and incubated with primary antibodies directed against ACE2 (1:100, abcam, cat: ab15348) and Ki-67 (1:100, MIB-1, Dako) in an Autostainer Link48 automated staining platform (Dako) for 20 min at room temperature. The slides were then washed in TBS and visualization was performed using the EnVision FLEX system (Dako) according to the manufacturer's instructions.

Immunofluorescent staining

Frozen cryosections of skin from patients and mice with the indicated genotype or treatment were fixed in ice-cold acetone for 10 min before staining. The following antibodies were used for immunofluorescent staining, Goat Anti-Rabbit IgG H&L AlexaFluor 488 (abcam， Cat：ab150077)，Rabbit polyclonal to Angiotensin Converting Enzyme 2 (abcam，Cat：ab15348)．4',6-Diamidino-2-phenylindole (DAPI) (Fluka) was used for the staining of nuclei. Antibodies were diluted in antibody diluent (Dako). Immunostained samples were analyzed with a fluorescence microscope (Olympus DP72). Isotype IgGs were used as control stainings.

Skin barrier dysfunction model

Mice (4-6 wks) were used in building the model. Psoriasis mouse model was established by topically treating each ear with 10 mg of 5% imiquimod cream (Imiquimod Cream 5%; 3M Pharmaceuticals) daily for 5 days. Atopic dermatitis mouse model were established by applying 3 nmol MC903 (MedChemExpress, Cat. No.: HY-10001) each ear per day for 10 days. Mechanical barrier disruption mouse model was achieved by stripping both sides of the earlobe with cellophane tape (3M Schotch) seven times. Contact dermatitis mouse model was established by applying 10ul 0.15% DNFB (Sigma-Aldrich, Inc., St. Louis, MOon the ear in day 0. 5 days later the ear was rechallenged by 10ul 0.15% DNFB and contact dermatitis like skin was induced.

Quantitative RT-PCR

RNA extraction and PCR were carried out as follows . Primer sequences used were designed for murine ACE2 FORWARD: TGGGCAAACTCTATGCTGACTG, murine ACE2 BACKWARD: CCCTTCATTGGCTCCGTTTCT. Murine GAPDH FORWARD: CCTCGTCCCGTAGACAAAATG GAPDH BACKWARD: TGAGGTCAATGAAGGGGTCGT. Murine β-actin FORWARD: AGCCATGTACGTAGCCATCC β-actin BACKWARD: CTCTCAGCTGGTGGTGAA. Human ACE2 FORWARD: CATTGGAGCAAGTGTTGGATCTT, human ACE2 BACKWARD: GAGCTAATGCATGCCATTCTCA. Human GAPDH FORWARD: CACATGGCCTCCAAGGAGTAA

HUMAN GAPDH BACKWARD: TGAGGGTCTCTCTTCCTCTTGT. Human CD209 FORWARD: GGATACAAGAGCTTAGCAGGGTG HUMAN CD209 BACKWARD: GCGTGAAGGAGAGGAGTTGC.

Reproduction of FITC conjugated S-protein and protein applying method

Dissolve proteins and FITCs in FluoroTag™ FITC Conjugation Kit（Sigma-Aldrich）kit buffer. FITC was slowly added to the recombinant novel coronavirus spike glycoprotein (Cusabio, cat: CSB-EP3324GMY1) while stirring. Than the mixture was coverred with foil and stir at room temperature for 2 hours. After that FITC conjugated protein was collected through the G-25. FITC conjugated S-protein was than applied to the ear skin of mouse model with barrier dysfunction and group control. 24 hours later the treated part of skin was taken and underwent immunofluorescence observation.

Skin barrier recovery

Mice were applied with skin barrier recovery moisture (Linoleic acid-ceramide moisturizer) 5 days before processing the skin barrier deteriorating method. Skin barrier recovery moisture was applied 10mg each ear per day.

Supplementary Material

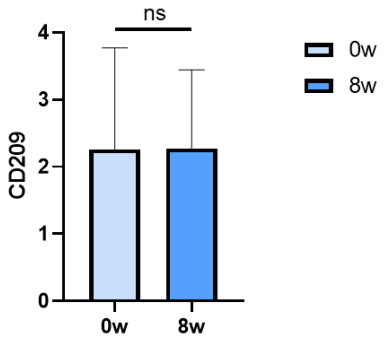

Fig. S1. **The expression of CD209 in the psoriatic lesion before and after the IL-17 antibody treatment.** Bars indicate mean±SD of experiment groups. Student's t test.

Fund：National Nature Science Foundation of China, 8183009591442123