# Project for elucidation of skin barrier mechanism

## Research overview

The skin is a structure that covers the surface of vertebrates and functions as a barrier to the outside world. In general, the structure that covers the surface of an individual organism and serves as a boundary between the outside world and the self is called the outer skin. The outer skin of vertebrates is the skin. The skin consists of the epidermis, dermis, and lipid tissues. The epidermis is a keratinized stratified squamous epithelium that consists of four layers, in order from the outside: the stratum corneum, stratum granulosum, stratum spinosum, and stratum basale. The outermost layer, the stratum corneum, is a very stable structure that can withstand dryness and physical force. The cells in the inner stratum granulosum are called SG1, SG2, and SG3 cells in order from the outside. Of these, only SG2 cells have a structure called tight junctions (TJs) that seal the cells together. The mammalian epidermis is equipped with a double barrier to withstand physical stimuli from the outside world and prevent the entry of pathogens and allergens. The keratinocyte barrier consists of keratinocytes and intercellular lipids that fill the gaps between the keratinocytes, and the TJ barrier consists of SG2 cells and TJs that seal the gaps between the cells.

These skin barriers have two main roles: one is the inner to outer barrier function that restricts the movement of water from the inside to the outside so that the skin can survive in the air without drying out. The other is the outer to inner barrier function that prevents invasion of pathogens and allergens. However, until now, it has been largely unclear what substances and to what extent the keratin and TJ barriers interact, and where and how allergens and pathogens that cross the barriers are captured by antigen-presenting cells of the immune system (namely, the epidermal Langerhans cells and dermal dendritic cells). In 2006, a new study was conducted on atopic dermatitis. On the contrary, in 2006, genetic mutations in filaggrin, a major constituent of the stratum corneum, were reported as a factor in the development of atopic dermatitis. This finding suggests that antigens entering the skin due to the breakdown of the stratum corneum barrier may be the cause of the onset of atopic dermatitis. In this study, we developed a new technique to visualize the skin barrier function and analyze how antigens that pass through the skin barrier are captured by antigen-presenting cells. In addition, by identifying causative genes from diseases and mouse models that show abnormalities in the stratum corneum, we identified genes that are important for skin barrier formation and analyzing the mechanism of abnormal stratum corneum formation. Through the development and analysis of filaggrin-deficient mice, mice with abnormal stratum corneum formation, and mice with skin TJ disorders, we have clarified the interaction and role of the stratum corneum and TJ barriers. We are now analyzing the barrier function in maintaining skin homeostasis, preventing invasion of pathogens and allergens, as well as the immune response to these invaders.

## Main results

1. To the best of our knowledge, this is the first report of three-dimensional observation of the TJ barrier in the skin, and have revealed that epidermal Langerhans cells actively capture antigens and allergens that pass through the stratum corneum by extending dendrites outside the TJ barrier (J Exp Med 2009). Through this elaborate mechanism, preemptive immunity against bacterial toxins can be established (J Exp Med 2011). We also found that the number of Langerhans cells, which are activated to extend dendrites outside the TJ barrier, is increased in atopic dermatitis (JACI 2014). This mechanism is thought to be one of the “transdermal sensitization mechanisms to allergens” that cause atopic dermatitis, food allergies, and asthma (J Clin Invest 2012).

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[Press Release] Elucidation of the skin's sophisticated barrier mechanism: New insights into the pathogenesis of skin diseases such as atopic dermatitis.

## Movie 1

https://www.youtube.com/watch?v=bCZx74MqafA
Tight junction barriers (green honeycomb pattern) and Langerhans cells (red cells with blue dendrites extending in all directions) in mouse ear skin. Images were obtained from three-dimensional observation of the entire mouse ear epidermis using a confocal microscope.

## Movie 2

https://www.youtube.com/watch?v=R6MTYf02vmA
Transdermal sensitization mechanism involving Langerhans cells. Activated Langerhans cells extend their dendrites outside the tight junction barrier to capture foreign antigens.

2. When the TJ barrier of the skin is observed in three dimensions, it is found that the TJs have a honeycomb-like mesh pattern. Most of the hexagons that make up this mesh pattern are single-layered, but approximately one in ten hexagons is double-layered (refer to Movie 1 above). Why are there double hexagons in some places? We discovered that these double hexagons are the secret of a metabolic mechanism that replaces cells with new ones without breaking the barrier (Elife 2016).
Press Release] Clarifying the Mechanism of Skin Metabolism and Barrier Maintenance - Cell Shapes Reveal the Secret of Maintaining Fresh Skin (click here)

## Movie 3

https://www.youtube.com/watch?v=gc5dwnFI9O0&t=3s
This movie shows how cells are replaced one after another while maintaining the barrier. The green lines between the cells are tight junctions that seal the gaps between the cells. In the stratified epithelium, only one layer of cells has tight junctions, so the green line is a single layer of meshwork. Only when the cells are replaced do the original green hexagons and the newly formed purple hexagons form a double hexagon. This mechanism allows cells to be replaced without any leakage of the tight junction barrier.

## Publications

1. Yokouchi M, Atsugi T, Logtestijn M, Tanaka R, Kajimura M, Suematsu M, Furuse M, Amagai M, Kubo A*. Epidermal cell turnover across tight junctions based on Kelvin's tetrakaidecahedron cell shape. Elife. 5:e19593, 2016.
https://pubmed.ncbi.nlm.nih.gov/27894419

2. Yokouchi M, Kubo A*, Kawasaki H, Yoshida K, Ishii K, Furuse M, Amagai M. Epidermal tight junction barrier function is altered by skin inflammation, but not by filaggrin-deficient stratum corneum. J Dermatol Sci. 77:28-36, 2015.
https://pubmed.ncbi.nlm.nih.gov/25511077

3. Yoshida K, Kubo A*, Fujita H, Yokouchi M, Ishii K, Kawasaki H, Nomura T, Shimizu H, Kouyama K, Ebihara T, Nagao K, Amagai M. Distinct behavior of human Langerhans cells and inflammatory dendritic epidermal cells at tight junctions in patients with atopic dermatitis. J Allergy Clin Immunol. 134:856-864, 2014.
https://pubmed.ncbi.nlm.nih.gov/25282566

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4. Kubo A*, Ishizaki I, Kubo A, Kawasaki H, Nagao K, Ohashi Y, Amagai M. The stratum corneum comprises three layers with distinct metal-ion barrier properties. Sci Rep. 3:1731, 2013.
https://pubmed.ncbi.nlm.nih.gov/23615774

5. Sasaki T, Shiohama A, Kubo A, Kawasaki H, Ishida-Yamamoto A,Yamada T, Hachiya T, Shimizu A, Okano H, Kudoh J, Amagai M: A homozygous nonsense mutation in the gene for Tmem79, a component for the lamellar granule secretory system, produces spontaneous eczema in an experimental model of atopic dermatitis. J Allergy Clin Immunol. 132:1111-1120 e1114, 2013.
https://pubmed.ncbi.nlm.nih.gov/24060273

7. Kubo A*, Nagao K, Amagai M. Epidermal barrier dysfunction and cutaneous sensitization in atopic diseases. J Clin Invest. 122:440-447, 2012.
https://pubmed.ncbi.nlm.nih.gov/22293182

8. Kawasaki H, Nagao K, Kubo A, Hata T, Shimizu A, Mizuno H, Yamada T, Amagai M. Altered stratum corneum barrier and enhanced percutaneous immune responses in filaggrin-null mice. J Allergy Clin Immunol. 129:1538-1546, 2012.
https://pubmed.ncbi.nlm.nih.gov/22409988

9. Ouchi T, Kubo A, Yokouchi M, Adachi T, Kobayashi T, Kitashima D, Fujii H, Clausen B, Koyasu S, Amagai M, Nagao K. Langerhans cell antigen capture through tight junctions confers preemptive immunity in experimental staphylococcal scalded skin syndrome. J Exp Med. 208:2607-2613, 2011.
https://pubmed.ncbi.nlm.nih.gov/22143886

10. Kubo A*, Nagao K, Yokouchi M, Sasaki H, Amagai M. External antigen uptake by Langerhans cells with reorganization of epidermal tight junction barriers. J Exp Med. 206:2937-2946, 2009.
https://pubmed.ncbi.nlm.nih.gov/19995951