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David Bending, PhD, Masahiro Ono, MD, PhD



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Interplay between skin barrier and immune cells in atopic dermatitis unravelled by mathematical modellingDavid Bending, PhD¹ & Masahiro Ono, MD PhD^{1§}

Department of Life Sciences, Faculty of Natural Sciences, Imperial College London, Sir Alexander Fleming Building, Imperial College Road, London, SW7 2AZ, United Kingdom

[§] Corresponding author: Masahiro Ono, MD PhD, Department of Life Sciences, Sir Alexander Fleming Building, Imperial College Road, South Kensington, London, SW7 2AZ, United Kingdom. E-mail address: m.ono@imperial.ac.uk

Atopic dermatitis (AD) is a chronic inflammatory skin disease, involving skin barrier impairment¹ and immune system dysregulation². The skin barrier is integral for the protection from microbe and allergen infiltration. It is physically in the outermost layer of the epidermis, and comprises terminally differentiated, denucleated keratinocytes, which structure is dependent on keratin and filaggrins (FLG), and extracellular matrix containing lipids, structural proteins, and serine proteases kallikreins (KLKs)¹. Dysfunctions of these components can result in barrier defects¹, as typically found in loss-of-function mutations of the *FLG* gene². In addition, the barrier function is regulated by the microbiota. For example, *Staphylococcus aureus* activates KLKs, which degrade FLG proteins³. Barrier disruption increases skin permeability to allergens, leading to innate immune cell activation (e.g. dendritic cells (DCs) and Langerhans cells), and subsequent priming of T-cells. T-cells play central roles, differentiating into T-helper type 2 (Th2) cells, which further disrupt barrier function⁴. Barrier disruption also directly activates keratinocytes through KLKs, which activate protease-activated receptor (PAR) 2 on keratinocytes¹, leading to the secretion of the cytokines IL-25, IL-33, and thymic stromal lymphopoietin (TSLP)², which promote Th2 differentiation².

AD represents a challenging disease to study mechanistically, given that the interplay of different cellular systems, environmental stressors and genetic variability is highly dynamic and complex. In the study by Domínguez-Hüttlinger *et al.*⁵, the multidisciplinary group took a new approach to generate and analyse a novel mathematical model of AD, and they investigated the systems mechanisms behind disease pathogenesis. Here we discuss their findings, critically analyse the paper, and investigate its biological and medical significance.

It is a critical issue in systems studies to determine the key components to be included in the model, which depends heavily on the aims of the study⁶. Here the authors investigated a minimal “skeletal” structure of the whole skin system, including barrier, keratinocytes, DCs and T-cells, aiming to understand how the activities of these different players are regulated in normal conditions, and dysregulated in AD. Based on a literature review, the authors chose barrier dysfunction, keratinocyte activation, and Th2 differentiation as the critical players to be analysed in their model, while also considering several mechanisms such as microbial invasion and antimicrobial peptides. Since microbiota were not investigated, the study is essentially about the interplay between two cellular systems: keratinocytes and immune cells.

Here we give an overview of the new mathematical model of AD by Domínguez-Hüttlinger *et al.*⁵. Skin barrier defects, which can be induced by environmental stressors, may result in increased microbial invasion (designated as “*infiltrated pathogens*” by the authors, although these presumably also include commensal bacteria) of the epidermal layer, further damaging the barrier integrity. Meanwhile, invading microbes convey barrier invasion through activation of keratinocytes via pattern-recognition receptors (PRRs) and indirectly PAR2 as well through KLKs. Microbes must be cleared by the activity of keratinocytes and immune cells, in order to return keratinocytes to the normal state. This mechanism is designated as a *reversible switch* (the authors call it *innate immune receptor activity*, however the mechanism is principally focused on *PRR activity in keratinocytes*). Meanwhile, stimulated keratinocytes activate DCs presumably by cytokines, while DCs act as a gatekeeper for the T-cell system: they retain and integrate the signals from keratinocytes, and only when the signals reach a certain threshold, DCs are able to activate T-cells, which the authors model as an irreversible differentiation of T-cells into allergy-causing Th2 cells. This is defined as an *irreversible switch* (**Fig. 1**). The two key switches in the keratinocyte system and the immune system were thus assumed to produce simple binary response (i.e. either ON or OFF) in their model.

Using their mathematical model, Domínguez-Hüttinger *et al.*⁵ performed *in silico* analyses, with the aim of revealing how the system behaves over time. In other words, the authors performed computational experiments, in which they experimented on virtual human skin and tested numerous clinical conditions and modelled the output activities of key players. To model the effects of genetic defects in skin barrier or immune cell function on barrier dysfunction, the authors titrated the parameters for barrier permeability and the clearance rate for epidermis-invading microbes *in silico*. This revealed that barrier integrity and keratinocyte activation had four distinct dynamic behavioral patterns. With no defects, a quick recovery to the healthy state is achieved (designated *Recovery*). However, with defects in both skin barrier and immune activation, the barrier integrity was rapidly lost and remains disrupted for a long time, leading to *Chronic Damage*. When just the clearance rate was reduced, modeling immune cell genetic defects, the imaginary skin showed two phenotypes: either the pattern of disruption and subsequent slow-recovery or that of chronic damage (designated *Bistability*). Interestingly, modeling skin barrier defects alone produced frequent intermittent immune activity but was computationally indistinguishable from that of the healthy steady state (designated *Oscillation*). The model behaviours were shown to be profoundly dependent on the two switches in keratinocytes and immune cells, which have been identified as the key presumptive mechanisms and highlighted as the “Double-Switch” in the paper.

Given the assumptions in their model, it is impressive that it successfully captured the common dynamics of inflammation in AD skin. Given that the mathematical model was constructed by “fitting” existing data to the model, either by using statistical regression or simply picking values from the literature, interrogation by independent experimental data is crucial for model validation. Here Domínguez-Hüttinger *et al.* analysed RNA-seq data of whole skin samples from keratinocyte-specific *Stat3* conditional knockout (KO) (*K5-Cre:Stat3^{fllox/fllox}*) mice, some of which spontaneously develop dermatitis. They showed that the expressions of NF- κ B target genes (the authors’ readout for environmental insults) were elevated in mice with AD symptoms compared to the asymptomatic mice. Whilst these data are consistent with the model, further experimentation would be required to disentangle cause from effect, as increased NF- κ B activity would not be an unexpected finding in inflamed skin.

The Double-Switch model states that inflammation in lesional skin becomes chronic once Th2 differentiation has occurred, while barrier dysfunction can be reversible if T-cells remain ‘naïve’. While this is the central part of their model, there are some additional complexities to be considered. For example, T-cell behavior is highly dynamic and some potential confounders are not included, notably T-cell plasticity and the loss of Th2 phenotype⁷, other T-cell differentiation programmes such as Th22⁸, and negative regulatory mechanisms through Foxp3⁹. In addition, their model does not really consider allergen sensitization. Here we suggest that the true irreversible switch in the T-cell system may be the generation of tissue-resident memory T-cells from skin-infiltrating T-cells upon antigen recognition¹⁰. Additionally, the authors used IgE secretion, an indirect readout for Th2 cells. Further work studying the dynamics of T-cell expression of Gata-3, Th2 cytokines (e.g. IL-4) and memory markers will no doubt further test the robustness of the Gata3 model component.

Although the proposed Double-Switch hypothesis would benefit from further experimental validation, the study should be praised for employing a rare, multidisciplinary approach to AD, providing a novel model framework for future investigation of skin disease dynamics. Furthermore, predicting treatment responses is a target area for future personalized medicine², and the study by Domínguez-Hüttinger *et al.* has shown that *in silico* analysis can be used to predict skin responses. With the rise of such a new approach, here we emphasize that the validity of mathematical models is dependent on *experimental* investigations⁶. This will require the development of new methods to reveal dynamic molecular and cellular activities *in vivo*, in order for Systems Medicine to break further new ground in understanding and treating diseases, such as AD.

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Figure Legend

Fig. 1. Schematic presentation of the Double-Switch model of Atopic Dermatitis proposed by Domínguez-Hütinger *et al*⁵. The authors analysed the literature and constructed a mathematical model with which they performed in silico experiments and assessed the dynamics of the skin and immune systems over time, testing innumerable conditions by changing model parameters. These investigations led to the establishment of the *Double-switch model*. The defect of skin barrier allows microbial invasion of the epidermal layers, which activates pattern-recognition receptor (PRR, depicted by yellow star) in keratinocytes. This activation status is reversible (Reversible Switch). The activation of keratinocytes is conveyed to dendritic cells (DC), which promotes the differentiation of naïve T-cells into Th2 cells. Th2-differentiation is considered irreversible in the model (Irreversible Switch). Differentiated Th2 cells further dysregulate keratinocyte functions.

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