Interplay between the skin barrier and immune cells in patients with atopic dermatitis unraveled by means of mathematical modeling

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Key words: *Atopic dermatitis, mathematical models, double switch, disease progression, disease phenotypes, preventive treatment*

Atopic dermatitis (AD) is a chronic inflammatory skin disease involving skin barrier impairment[1] and immune system dysregulation.[2] The skin barrier is integral for the protection from microbe and allergen infiltration. It is located physically in the outermost layer of the epidermis and comprises terminally differentiated denucleated keratinocytes, the structure of which is dependent on keratin and filaggrin (FLG), and extracellular matrix containing lipids, structural proteins, and the serine protease subgroup kallikreins (KLKs).[1] Dysfunction of these components can result in barrier defects,[1] as typically found in loss-of-function mutations of the *FLG* gene.[2]

In addition, barrier function is regulated by the microbiota. For example, *Staphylococcus aureus* activates KLKs, which degrade FLG proteins.[3] Barrier disruption increases skin permeability to allergens, leading to innate immune cell activation (eg, dendritic cells [DCs] and Langerhans cells) and subsequent T-cell priming. T cells play central roles, differentiating into T_H2 cells, which further disrupt barrier function.[4] Barrier disruption also directly activates keratinocytes through KLKs, which activate protease-activated receptor 2 on keratinocytes,[1] leading to secretion of the cytokines IL-25, IL-33, and thymic stromal lymphopoietin,[2] which promote T_H2 differentiation.[2]

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-Hüttinger et al,[5] the multidisciplinary group took a new approach to generate and analyze a novel mathematical model of AD, and they investigated the systems mechanisms behind disease pathogenesis. Here we discuss their findings, critically analyze the article, 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 depend heavily on the aims of the study.[6] Here the authors investigated a minimal "skeletal" structure of the whole skin system, including the barrier, keratinocytes, DCs, and T cells, aiming to understand how the activities of these different players are regulated in normal conditions and dysregulated in patients with AD. Based on a literature review, the authors chose barrier dysfunction, keratinocyte activation, and T_H2 differentiation as the critical players to be analyzed in their model while also considering several mechanisms, such as microbial invasion and antimicrobial peptides. Because microbiota were not investigated, the study is essentially about the interplay between 2 cellular systems: keratinocytes and immune cells.

Here we provide an overview of the new mathematical model of AD by Domínguez-Hüttinger et al.[5] Skin barrier defects, which can be induced by environmental stressors, can 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 through pattern recognition receptors and indirectly through protease-activated receptor 2, as well through KLKs. Microbes must be cleared by the activity of keratinocytes and immune cells 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 pattern recognition receptor activity in keratinocytes). Meanwhile, stimulated keratinocytes activate DCs, presumably through cytokines. 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 are DCs able to activate T cells, which the authors model as an irreversible differentiation of T cells into allergy-causing T_H2 cells. This is defined as an "irreversible switch" (Fig 1). The 2 key switches in the keratinocyte and immune systems were thus assumed to produce simple binary response (ie, 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 modeled the output activities of key players. The authors titrated the parameters for barrier permeability and the clearance rate for epidermis-invading microbes *in silico* to model the effects of genetic defects in the skin barrier or immune cell function on barrier dysfunction. This revealed that barrier integrity and keratinocyte activation had 4 distinct dynamic behavioral patterns. With no defects, a quick recovery to the healthy state is achieved (designated recovery). However, with defects in both the skin barrier and immune activation, 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

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M.O. is a Biotechnology and Biological Sciences Research Council (BBSRC) David Phillips Fellow.

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflict of interest.

Received for publication January 23, 2017; revised March 7, 2017; accepted for publication March 27, 2017.

Available online April 7, 2017.

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J Allergy Clin Immunol 2017;139:1790-2.

0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2017.03.015

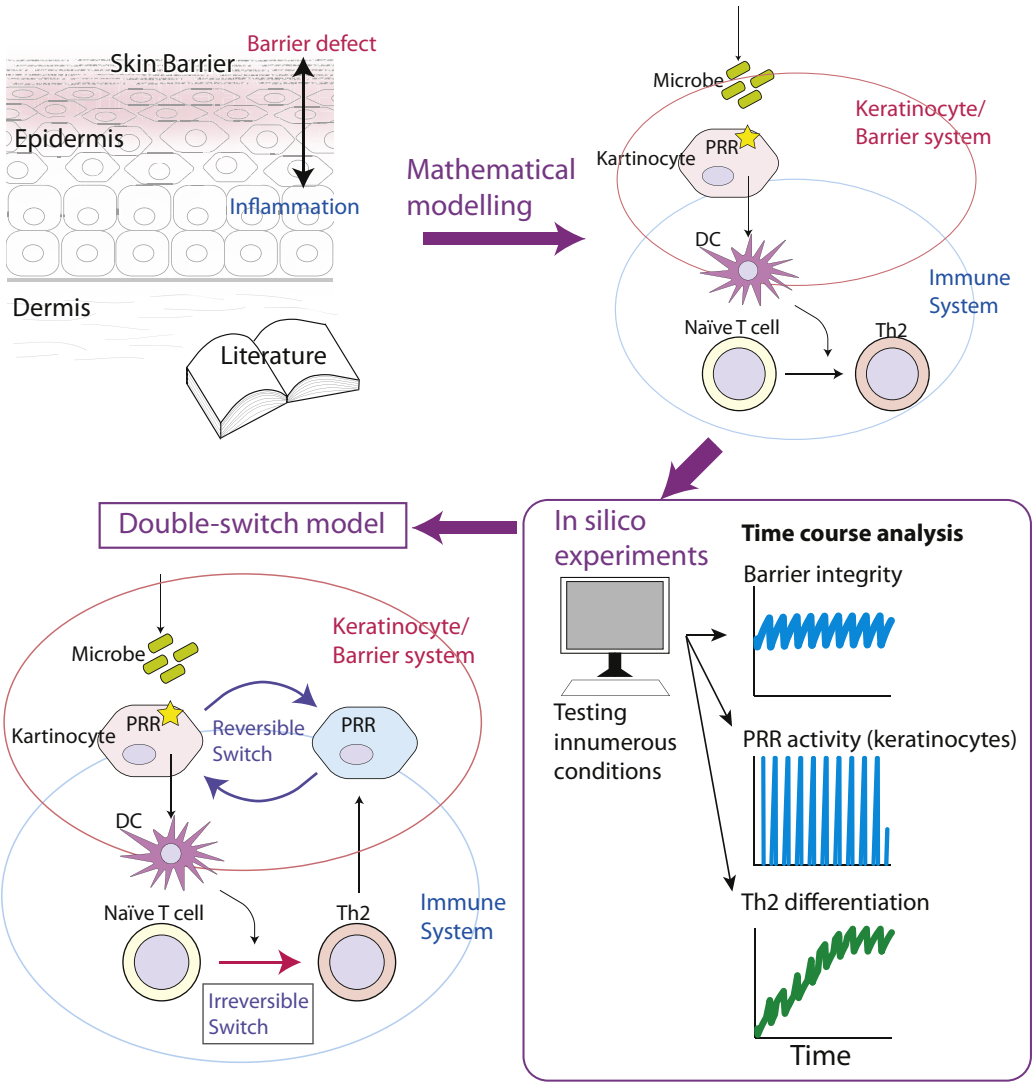

FIG 1. Schematic presentation of the double-switch model of AD proposed by Domínguez-Hüttinger et al.[5] The authors analyzed the literature and constructed a mathematical model with which they performed *in silico* experiments and assessed the dynamics of the skin and immune systems over time, testing innumerous conditions by changing model parameters. These investigations led to establishment of the double-switch model. The skin barrier defect allows microbial invasion of the epidermal layers, which activates pattern recognition receptors (*PRR*; depicted by *yellow stars*) in keratinocytes. This activation status is reversible (reversible switch). The activation of keratinocytes is conveyed to DCs, which promote the differentiation of naive T cells into T_H2 cells. T_H2 cell differentiation is considered irreversible in the model (irreversible switch). Differentiated T_H2 cells further dysregulate keratinocyte functions.

2 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 behaviors were shown to be profoundly dependent on the 2 switches in keratinocytes and immune cells, which have been identified as the key presumptive mechanisms and highlighted as the "double-switch" in the article.

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[5] analyzed RNA sequencing data of whole skin samples from keratinocyte-specific signal transducer and activator of transcription 3 *(Stat3)* conditional knockout *(K5-Cre:Stat3$^{flox/flox}$)* mice, some of which have spontaneous dermatitis. They showed that expression of nuclear factor κB target genes (the authors' readout for environmental insults) were increased in mice with AD symptoms compared with that in asymptomatic mice. Although these data are consistent with the model, further experimentation would be required to disentangle cause from effect because increased nuclear factor κB activity would not be an unexpected finding in inflamed skin.

The double-switch model states that inflammation in lesional skin becomes chronic once T_H2 differentiation has occurred, whereas barrier dysfunction can be reversible if T cells remain naive. Although 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 loss of the T_H2 phenotype[7]; other T-cell differentiation programs, such as T_H22[8]; and negative regulatory mechanisms through forkhead box p3.[9] In addition, their model does not consider allergen sensitization. Here we suggest that the true irreversible switch in the T-cell system might be generation of tissue-resident memory T cells from skin-infiltrating T cells on antigen recognition.[10] Additionally, the authors used IgE secretion, an indirect readout for T_H2 cells. Further work studying the dynamics of T-cell expression of GATA-3, T_H2 cytokines (eg, IL-4), and memory markers will no doubt further test the robustness of the GATA-3 model component.

Although the proposed double-switch hypothesis would benefit from further experimental validation, the study should be praised for using a rare multidisciplinary approach to AD, providing a novel model framework for future investigation of skin disease dynamics. Furthermore, predicting treatment response is a target area for future personalized medicine,[2] and the study by Domínguez-Hüttinger et al[5] has shown that *in silico* analysis can be used to predict skin responses. With the increase in such a new approach, we emphasize that the validity of mathematic models is dependent on experimental investigations.[6] This will require the development of new methods to reveal dynamic molecular and cellular activities *in vivo* for systems medicine to break further new ground in understanding and treating diseases such as AD.

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