Multi-scale modelling of epithelium homeostasis

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Most of the biological processes in physiological systems are tightly controlled by regulatory interactions across different temporal and spatial scales. For example, while epithelium homeostasis is achieved by the orchestration among growth, differentiation and apoptosis of different cell types, the phenotype of each cell within the epithelium is determined by the concentrations of local effectors, such as enzymes and gene transcripts. The concentrations of these effectors are regulated at the cellular level by stimulus–induced, fast reacting protein-protein interaction (PPI) and genetic networks. In turn, stimulus concentration depends on the epithelial permeability, a tissue level property that is determined by epithelium homeostasis. Malfunction in these multi-scale feedback regulatory networks are often triggered by combinations of environmental and genetic risk factors, and can lead to the loss of epithelial homeostasis, inflammation and thus the development of diseases such as asthma [2], Chron's disease [3] and Atopic dermatitis (AD) [3]. Understanding of the underlying mechanisms of development of these pathological conditions benefits from an appropriate mathematical modelling framework, since the epithelium homeostasis is achieved through highly interconnected, complex regulatory networks at different scales.

Here, we propose a mathematical model of AD, a skin disease affecting nearly 30% of the paediatric population worldwide [6]. AD is characterized by a loss of homeostasis in the uppermost epidermal layer (skin barrier) and by an exacerbated immunological response to environmental stimuli. AD is also associated to other allergic diseases, such as asthma and allergic rhinitis. Although the causes of AD remain unknown, several environmental and genetic risk factors have been identified [5]. With our model, we aim to understand essential regulatory mechanisms underlying skin barrier homeostasis and inflammation leading to AD. This will contribute not only to identify deregulation leading to AD, but also broaden the general understanding of other pathophysiological processes related to the loss of epithelium homeostasis.

We model essential regulatory interactions at cellular and tissue levels that are necessary to reproduce the qualitative features of AD, i.e. hypersensitivity to environmental stimuli and loss of skin barrier integrity. Skin barrier prevents the exacerbated penetration of environmental stimuli into the inner epidermal layers. Healthy skin barrier integrity is achieved by an appropriate balance between the production and desquamation rates of terminally differentiated keratinocytes and by the maintenance of a high lipid content. Over-activation of proteolytic enzyme Kallikrein (KLK) leads to increased skin barrier desquamation by degrading the inter-cellular junctions of the skin barrier. Moreover, KLK also inhibits the lamellar body lipid release into the skin barrier, by activating its target Protease Activated Receptor (PAR2), further weakening the skin barrier integrity. Defective skin barrier allows more exogenous stimulus to invade into the inner epidermal layers, triggering the cellular regulatory PPI networks and the immune system. We denote the penetrated concentration of stimulus at the inner epidermal layers as $\boldsymbol{S_{in}}$. The activation of PAR2 also induces inflammation, which in turn leads to the eradication of the accumulated $\boldsymbol{S_{in}}$.

The cellular level PPI network regulating KLK activity is induced by $\boldsymbol{S_{in}}$, which, together with activated PAR2, triggers the expression and release of KLK and its inhibitor LEKTI into the extra cellular space. Auto-activated KLK is then either inhibited by LEKTI or activates PAR2 and KLK by proteolysis (Figure 1A). These enzymatic interactions occur at much faster time-scale than the tissue level dynamics. Therefore, we represented this multi-scale system of regulatory interactions by a system of integral–differential equations with two different time scales.

To investigate the role of different risk factors in the development of AD, our model incorporated four main genetic and environmental elements known to predispose for AD: Under-expression of LEKTI (which we call AD-LEKTI condition), increased catalytic activity of KLK due to an increase in the epidermal pH (AD-pH condition), decreased capacity of the immune system to eradicate antigens (AD-

immune condition), and a chronic skin barrier dysfunction due to the lack of fillagrin (AD-fillagrin condition) (Figure 1B).

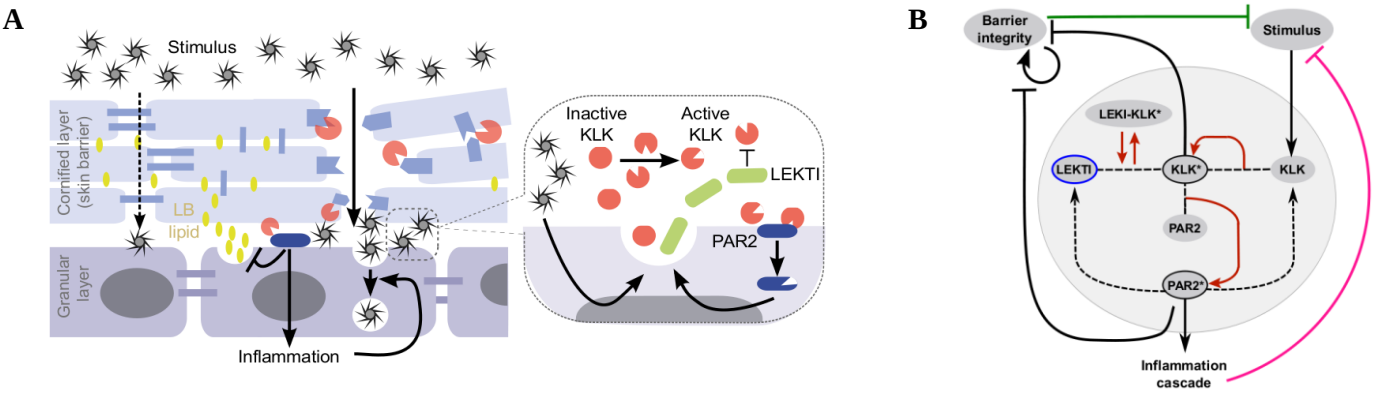

Figure 1. Tissue and cellular level KLK reaction networks regulating skin barrier homeostasis and inflammation. *A*: Cartoon representation of the multi-scale reaction network affecting skin barrier homeostasis and inflammation. *B*: Schematic representation of the reaction network. The fast reacting PPI network is represented inside the gray circle. The slow, tissue level interactions are represented by solid lines. The risk factors to be investigated are denoted by coloured lines; Blue: Under expression of LEKTI (AD-LEKTI); Red: Increased catalytic activity of KLK due to an increase in the epidermal pH (AD-pH); Magenta: Decreased capacity of the immune system to eradicate antigens (AD-immune); Green: Chronic skin barrier dysfunction due to the lack of fillagrin (AD-fillagrin).

To explore the fast reacting PPI network of our model, we performed a steady state analysis of the concentrations of PAR2*-induced inflammation as a function of S_{in}. The resulting bifurcation diagrams, corresponding to dose–response curves, exhibit the characteristic persistence of inflammation by bistability with a bistable region (r) spanned between the values of S_{in} leading to the onset (S^+) and stop (S^-) of the inflammatory response (Figure 2A). The risk factors AD-LEKTI and AD-pH, mimicking two different AD-conditions, lead to a more severe inflammatory response, as indicated by lower S^+ and higher r values, representing increased sensitivity to environmental stimuli and increased persistence of inflammation. Thus, our PPI model successfully captured exacerbated immunological response to environmental stimuli, typically found in AD patients (Figure 2B). Our findings showed a good agreement with microarray data representing the expression levels of PAR2 target genes in AD patients (Figure 2C) [1].

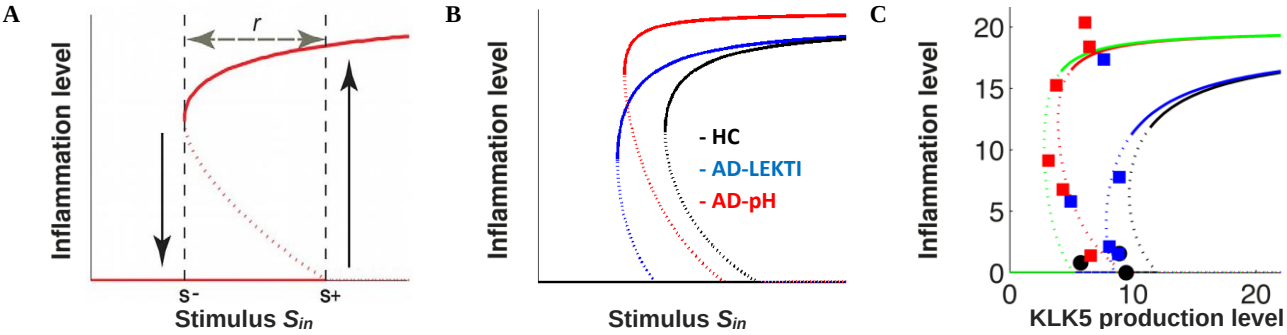

Figure 2: **Simulation results of the PPI network**. *A:* Outbreak and persistence of the inflammation exhibited by bistability in the bifurcation diagrams with stimulus level as a bifurcation parameter. The region of bistability (***r*),** spanned between the stimulus concentration leading to the onset (S^+) and stop (S^-) of inflammation, represents the persistence of the inflammation. *B:* AD conditions (AD-LEKTI, AD-pH) lead to an increased sensitivity to environmental stimuli and persistence of the inflammation, characteristic of AD patients. *C:* Agreement of the simulation results (lines) with microarray data (symbols) from AD patients. Black circles: Healthy control; Red squares: Lesional AD patients; Blue squares: Non-lesional AD patients. Black line: Healthy control; blue line: AD-LEKTI; Red line: AD-pH; Green line: AD-LEKTI-pH. Figures reproduced from [1].

We next incorporated the slow reacting, tissue level elements (skin barrier integrity, inflammation and S_{in}) by coupling the above mentioned fast reacting PPI module via the S_{in}–dependent steady state

concentrations of KLK* and PAR2* (Figure 1B). Our model exhibits three different types of dynamic responses in the skin barrier integrity to environmental challenges: complete recovery (homeostasis) observed in healthy individuals, periodic loss of homeostasis (oscillations) by moderately atopic patients, and chronic loss of homeostasis by severe AD patients (Figure 3A). We defined the index values τ^- and τ^+ to quantitatively characterize these three responses (Figure 3B**)**. τ^- is the time at which S_{in} decreases to a concentration S^- at which KLK and PAR2 are no longer active (Figure 2A), allowing the recovery of the skin barrier integrity and its eventual return to homeostatic level. τ^+ is defined as the time when S_{in} increases again up to a value S^+ at which PAR2 and KLK are reactivated (Figure 2A), leading to a recurrence of the skin barrier damage. Higher τ^- indicates slower recovery of the skin barrier integrity, and smaller τ^+ corresponds to more frequent recurrence of skin barrier damage. Our results strongly suggest a direct correlation between risk factors and qualitative behaviours. For instance, while AD-fillagin and AD-LEKTI lead to an oscillatory behaviour, AD-pH and AD-immune result in a chronic loss of homeostasis (Figure 3C). Our modelling framework also allows us to examine more complex relations between the combination of different risk factors, the qualitatively classified symptoms and the effect of different treatments, such as corticosteroids and emollients, by simulating a heterogeneous population of virtual patients, by relating their risk factor phenotype to the resulting index values and assessing the effect of the treatments on the qualitative behaviours.

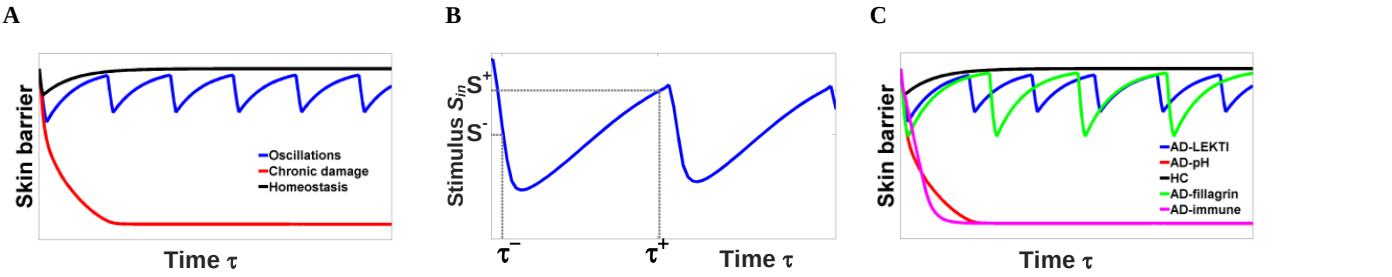

Figure 3: Simulation results of the skin barrier function. *A:* Three qualitatively different types of dynamic responses in the skin barrier integrity to environmental challenges: Complete recovery (homeostasis), periodic loss of homeostasis (oscillations) and chronic damage. *B*: Index values τ^- and τ^+ to quantitatively characterize the three responses: τ^- is the time when S_{in} decreases to a concentration S^- at which KLK and PAR2 are no longer active, allowing the recovery of the skin barrier integrity and its eventual return to homeostatic level. τ^+ is defined as the time when S_{in} increases again up to a value S^+ at which PAR2 and KLK are reactivated, leading to a recurrence of the skin barrier damage. Higher τ^- indicates slower recovery of the skin barrier integrity, and smaller τ^+ corresponds to more frequent recurrence of skin barrier damage. *C*: Relation between the known risk factors of AD and qualitative dynamic behaviours.

Our results contribute to the identification of key mechanisms underlying the regulation of skin barrier homeostasis and inflammation related to AD. We further proposed index values that can be used to quantify the severity of AD symptoms in the clinic. It will help for more accurate description and personalised treatment of the disease. Since this study captured the essential regulatory interactions in skin barrier homeostasis and inflammation, our results can be applied to understand the general principles underlying the regulation of epithelial homeostasis and inflammation. The proposed modelling approach, based on the time scale separation and modularization, provides a theoretical framework for studying multi-scale regulatory interactions, characteristic of nearly all physiological systems.

References

1. Tanaka RJ, Ono M, Harrington HA. PloS one. 2011; **6**(5):e19895.
2. Lambrecht BN, Hammad H. Lancet. 2010; **376**(9743):835–43.
3. Artis D. Nat. Rev. Immunol. 2008; **8**(6):411–20.
4. Elias PM, Schmuth M. Curr Allergy Asthma Rep. 2009; **9**:265–72.
5. Niebuhr M, Werfel T. Curr Opin Allergy Clin Immunol. 2010; **10**(5):463–8.
6. Sterne JAC, Golding J, Kennedy CTC, et al. Brit J Dermatol. 2003; **149**:1023–8.