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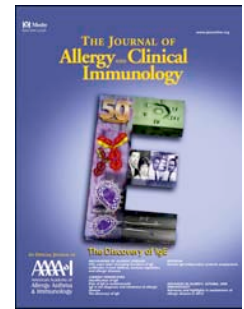
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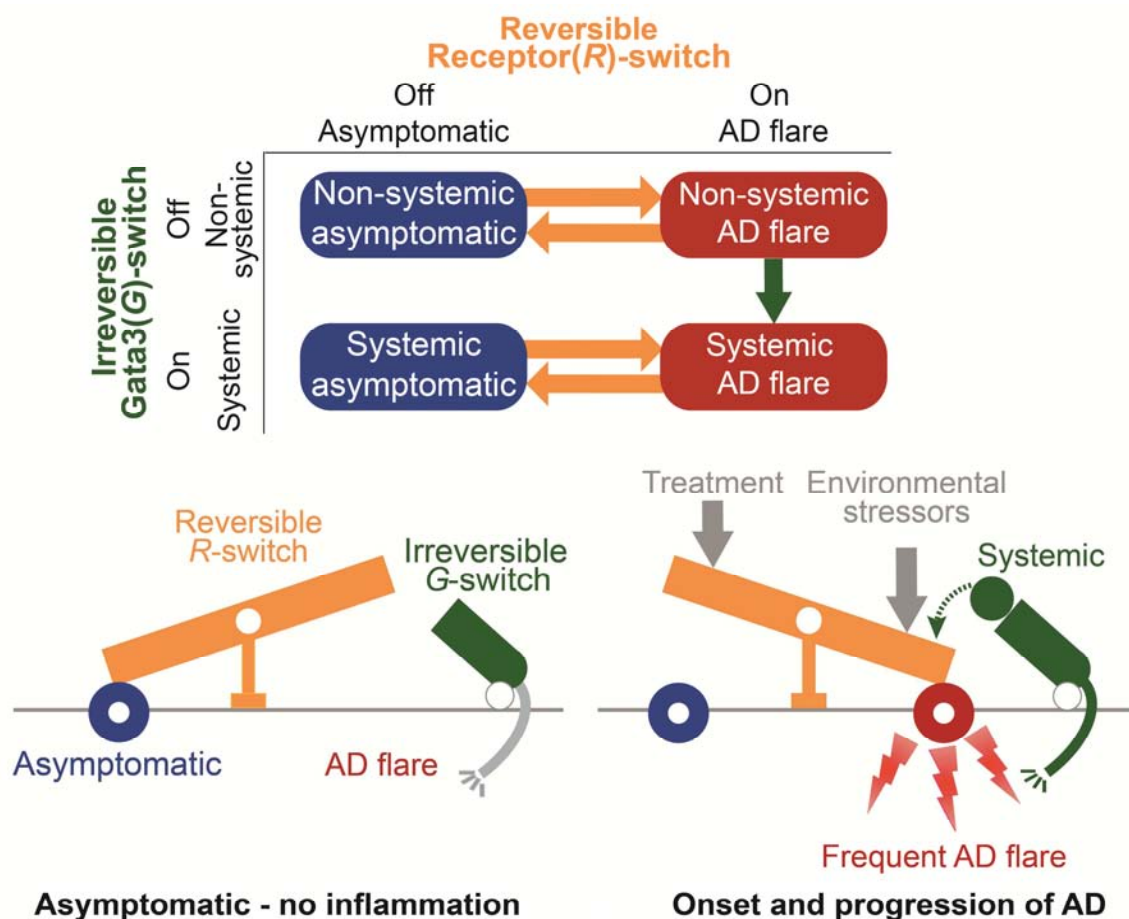
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Mathematical Modeling of Atopic Dermatitis Reveals “Double switch” Mechanisms Underlying Four Common Disease Phenotypes

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Abstract

Background: The skin barrier acts as the first line of defense against constant exposure to biological, microbial, physical and chemical environmental stressors. Dynamic interplay between defects in the skin barrier, dysfunctional immune responses, and environmental stressors are major factors in the development of atopic dermatitis (AD). A systems-biology modeling approach can yield significant insights into these complex and dynamic processes through integration of prior biological data.

Objective: To develop a multi-scale mathematical model of AD pathogenesis that describes the dynamic interplay between the skin barrier, environmental stress and immune dysregulation, and use it to achieve a coherent mechanistic understanding of onset, progression and prevention of AD.

Methods: We mathematically investigated synergistic effects of known genetic and environmental risk factors on the dynamic onset and progression of the AD phenotype, from a mostly asymptomatic mild phenotype to a severe treatment-resistant form.

Results: Our model analysis identified a “double switch”, with two concatenated bistable switches, as a key network motif that dictates AD pathogenesis: The first switch is responsible for the reversible onset of inflammation; The second switch is triggered by long-lasting or frequent activation of the first switch, causing the irreversible onset of systemic Th2 sensitization and worsening of AD symptoms.

Conclusions: Our mathematical analysis of the bistable switch predicts that genetic risk factors lower the threshold of environmental stressors to trigger systemic Th2 sensitization. This analysis predicts and explains four common clinical AD phenotypes from a mild and reversible phenotype through to severe and recalcitrant disease and provides a mechanistic explanation for clinically-demonstrated preventive effects of emollient treatments against development of AD.

Key messages

- Mathematical modeling integrating known pathogenic mechanisms discloses that a “double switch” motif provides a coherent explanation of the dynamic onset, progression and prevention of atopic dermatitis.
- This mathematical model provides a framework to investigate new and better treatments for atopic dermatitis.

Capsule summary

Mathematical models that integrate biological data can help explain complex pathogenic processes in AD, offering a coherent explanation of mechanisms behind four common AD phenotypes and the dynamic onset, progression, and prevention of AD.

Key words

Atopic dermatitis, mathematical models, double switch, disease progression, disease phenotypes, preventive treatment

Introduction

Atopic dermatitis (AD) is a common chronic skin disease, characterized by persistent skin inflammation and a defective skin barrier prone to infections¹. While AD affects up to 25% of children worldwide² with a continuous increase in the number of patients and treatment costs³, clear guidance and consensus for effective treatment strategies for prevention, and induction of remission, are yet to be fully established⁴. The scientific basis for recent clinical recommendations of "proactive therapy" is largely based on clinical trial data with limited duration and scope⁵, and waits to be further strengthened by a better understanding of the pathogenic mechanisms of AD. A system-level understanding of AD pathogenesis, however, may lie beyond the ethical and practical reach permitted by clinical and experimental studies, and could be supported by a systematic, extensive investigation of the disease dynamics using mathematical models^{6,7}.

Previous studies have identified genetic and environmental risk factors for AD onset including (1) skin barrier function (the *FLG* gene encoding profilaggrin⁸⁻¹¹ and 30 other putative loci for skin barrier function and the (2) immune system including innate and adaptive immunity^{12,13,14}. In addition, (3) microbiome composition has been shown to play a critical role^{15,16}. Each of these risk factors perturbs the system of strongly intertwined regulatory interactions between the keratinocytes (which along with the extracellular lipids provide the barrier function and also initiate many innate immune responses in the epidermis) and mediators of the adaptive immunity such as dendritic cells (DCs) and T cells. Perturbations triggered at one part of the system can propagate to another part through interactions, causing synergistic effects of risk factors and a gradual aggravation of the AD phenotype from a mostly asymptomatic mild phenotype at its early stage¹⁷ to a severe treatment-resistant form¹⁸. Understanding of this dynamic pathogenesis thus requires elucidation of the synergistic effects of multiple risk factors on AD pathogenesis

(e.g. mutations in skin barrier components accompanied by environmental insults) and the dynamic interplay between the skin barrier, immunity and environment, as suggested by several clinical^{14,19} and animal or *in vitro* experimental studies^{20–23}.

In this study, we develop a mathematical *in silico* model of AD pathogenesis that describes the complex and dynamic interplay between skin barrier function, immune responses, and environmental stressors, based on clinical and experimental data and our previous model of the early stage of AD²⁴. Through systematic, mathematical investigation of the synergistic effects of the risk factors on dynamic progression of AD pathogenesis, we tested the hypothesis that genetic risk factors predispose patients to AD progression and lower the threshold for environmental stressors to drive clinically severe AD²⁵. We uncover mechanisms underlying AD onset and progression, explore effective treatment strategies to prevent progression of AD, and answer three basic questions about onset, progression and prevention of AD: Why do some patients with genetic AD risk factors appear initially asymptomatic but then easily develop clinically severe AD? Why do some patients progress to severe AD, while the others stay with mild AD? What are the underlying mechanisms behind effective prevention of AD development by emollients in neonates, as demonstrated in recent clinical trials^{26,27}?

Results

Model overview

Our proposed mathematical model of AD pathogenesis (Fig. 1(A, B)) is a system-level representation of the prominent interactions between environmental stressors, skin barrier integrity and immune responses, that were identified based on the empirical evidence from numerous clinical^{11,20,28–30} and experimental *in vivo*^{22,31–35} or *in vitro*^{36–38} studies. The model is described by a hybrid system of three ordinary differential equations (ODEs) and includes a

double switch motif with two concatenated bistable switches as detailed below. The first switch is responsible for onset of AD flares and the second switch for progression to severe AD. The nominal values of the 21 model parameters were identified from the literature (Table I). This mathematical model includes only the major cellular and biochemical regulators required to describe a global regulatory structure and mechanisms underlying onset, progression and prevention of AD, rather than explicitly incorporating the fine details of the complex molecular and cellular processes that control epidermal function.

Our model results are validated by clinical data and experimental data derived from mouse models with perturbations in either skin barrier function, immune responses or environmental stressors, the interplay of which is investigated in our model: mouse models with barrier deficiency (flaky tail (*ft*) mice^{21,22} with the double mutation involving filaggrin gene (*flg*) and *Tmem79* which encodes lamellar granules³⁹, filaggrin null (*flg*^{-/-}) mice²³, and *Tmem79*-deficient mice^{40,41}), keratinocyte-specific *Stat3*-ko mice with dysregulated immune responses⁴²⁻⁴⁴, and mouse models in which AD symptoms are induced solely by environmental stressors^{45,46}.

A first switch for onset of AD flares

The first part of the model (processes a-e in Fig. 1(A, B) described below) elucidates the mechanisms of how the activity of the first switch (switch-like activation of innate immune receptors) is regulated and leads to AD flares.

Healthy barrier integrity is maintained by a combination of regulated processing of filaggrin²² and lipid contents that constitute lipid envelopes of corneocytes, and protects the body from environmental stressors (process a). When the barrier integrity is compromised by, for example, excessive skin desquamation via active kallikreins³¹, environmental stressors including pathogens such as *S. aureus*²⁸ infiltrate the barrier and stress the viable epidermis. They are

recognized by innate immune receptors, such as danger-associated molecular pattern (DAMP)- and pathogen-associated molecular pattern (PAMP)-recognizing toll-like receptors (TLRs) and protease-activated receptors (PAR2s)^{36,47} (process b). Activation of the innate immune receptors triggers innate immune responses to eradicate the infiltrated pathogens, for example via release of antimicrobial peptides (AMPs)⁴⁷ (process c), further activates kallikreins³⁷ (process d), interferes with the *de novo* production of skin barrier lipids³² (process e), and triggers inflammation³³, resulting in AD flares with increased levels of IL-33⁴⁸, TSLP³⁴ and other alarmin cytokines⁴⁹.

Activation of the innate immune receptors is modelled by a reversible bistable switch⁵⁰ between the off-and on-states ($R = R_{\text{off}}$ and R_{on}) with the activation (P^+) and inactivation (P^-) thresholds for the amount of environmental stressors, such as pathogen load, that are recognized by the innate immune receptors (Fig. 1 (C), *R*-switch). When the *R*-switch is on, clinical symptoms of AD flares appear as increased inflammation accompanied by decreased barrier integrity. Our model demonstrates that activation of the innate immune receptors and the subsequent onset, and resolution, of the AD flares is mainly regulated by a combination of negative and positive feedbacks via innate immune responses and barrier integrity (Fig. 1(B)). The relative strengths of the feedbacks can be inferred from the two most prominent genetic risk factors of AD: mutations in the *FLG* gene^{8,9} resulting in a higher skin barrier permeability²³, and a dysregulated expression of innate immune system components¹², including the regulators of AMP expression (such as TLRs⁵¹, nucleotide-binding oligomerization domain (NODs)¹³, and NF- κ B⁵²) leading to dysfunctional immune responses and a diminished capacity in eradicating the infiltrated pathogens.

Synergetic effects of genetic risk factors and environmental triggers on dynamic phenotypes for AD flares

We investigated the effects of these two genetic risk factors in our model by titrating the model parameters that quantify skin barrier permeability (κ_p) and the capacity to eradicate infiltrated pathogens (α_i) in Eq. (1). These analyses correspond to examining carriers of different variations of the genetic risk factors and observing how AD can be developed and proceeded in each cohort as a result of the synergistic effects of the environmental stressors and the two genetic risk factors. Our model analysis revealed the following four qualitatively different *dynamic phenotypes* (Fig. 2(A, B)) in response to environmental stressors (e.g. pathogenic challenges).

When these intrinsic genetic defects do not exist, a quick recovery of the system to the healthy steady state, with homeostatic barrier integrity and no AD flares ($R = R_{\text{off}}$), is achieved after a transient decrease in the barrier integrity and transient AD flares (*recovery dynamic phenotype*); simultaneous existence of these two genetic risk factors results in convergence of the system to the unhealthy steady state, corresponding to a chronically decreased barrier integrity and persistent AD flares ($R = R_{\text{on}}$) that potentially leads to chronic tissue damages (*chronic damage dynamic phenotype*); genetic defects leading to dysregulated immune responses result in bistability, where either the healthy or unhealthy steady state is achieved (*bistability dynamic phenotype*); genetic defects leading to high skin barrier permeability results in persistent oscillatory dynamics due to the switching of R between R_{on} and R_{off} (*oscillation dynamic phenotype*).

The steady state barrier integrity for the *oscillation dynamic phenotype* is computationally predicted to be indistinguishable to that of the healthy steady state (Fig. 2(C)), with a slightly lower mean but with an increased variability. These long-term dynamic behaviours are concordant with a slightly lower but more variable skin barrier integrity observed in $flg^{-/-}$ ²³ and ft

mice^{21,22}, compared to wild type (wt). Our model analysis further predicts gradual decrease of the steady state barrier integrity for increased barrier permeability (κ_P), corresponding to the dose-dependent effects of filaggrin deficiency on the severity of AD symptoms⁹ (Fig. 2 (D)).

The *bistability* dynamic phenotype may also be asymptomatic (or subclinical) if it converges to the healthy steady state. Our model analysis, however, demonstrates that the healthy steady state of the *bistability* dynamic phenotype is distinguishable from that of the *recovery* dynamic phenotype, by the much longer time for recovery to regain the same healthy steady state from transient barrier damage (Fig. 2(E)), due to the existence of the second stable (unhealthy) steady state. This model prediction is consistent with slower skin barrier recovery following tape stripping observed in non-lesional skin of AD patients compared with healthy individuals⁵³, and in inflamed compared with non-inflamed human skin⁵⁴.

The boundaries of the basins of attraction for the *bistability* dynamic phenotype are computationally obtained by finding the points of tangency between the switching thresholds for the reversible *R*-switch, P^+ and P^- and the $(P(t), B(t))$ -trajectories on the phase plane (Fig. 2 (F)).

The system moves from the healthy to the unhealthy steady state if external inputs (e.g. environmental aggressors) push the system into the unhealthy basin of attraction. This worsening of the state was observed in *ft* mice^{21–23}, as well as in *Stat3*-ko mice, which demonstrated AD symptoms only after challenged by haptens or lipopolysaccharides (LPS), an activating ligand of the innate immune receptor TLR4⁵⁵ (see Fig. E1 in the Online Repository). Likewise, the system can move back to the healthy from the unhealthy steady state if external inputs (e.g. treatments) bring the system back to the healthy basin of attraction, achieving remission. Once the remission is induced, maintenance therapy to keep the system in the healthy basin of attraction may achieve control of AD symptoms⁵⁶. This potential switching between the healthy and unhealthy steady states, by environmental aggressors or treatments, is a distinguished feature of the *bistability*

dynamic phenotype, and is not observable in the *recovery* and *chronic damage* dynamic phenotypes.

A second switch for progression of AD

The second part of the model (processes f-h in Fig. 1 (A, B) described below) includes the main biochemical and cellular players for the allergy-mediating (Th2) adaptive immune responses triggered by AD flares, and is a system-level synthesis of several lines of empirical evidences from clinical and experimental studies. Using this model, we elucidate mechanisms behind progression of AD to establish systemic Th2 sensitization (including elevated IgE, a marker of a Th2 skewed AD phenotype), and systematically investigate how the duration and frequency of AD flares affect the onset of systemic inflammation, including allergic inflammation.

Activation of the innate immune receptors cause AD flares with increased levels of cytokines including TSLP³⁴, alarmins such as IL-33⁴⁸ and others⁴⁹, which together contribute to activation of dendritic cells (DCs) in the epidermis³⁴. These activated DCs migrate to lymph nodes (process f), where they increase the levels of IL-4³⁵ and induce expression of Gata3³⁰ (process g), the master transcriptional regulator that controls the irreversible differentiation of naïve T cells into Th2 cells^{35,38}. Differentiated Th2 cells then migrate back to the epidermis, where they contribute to establish an allergic and pro-inflammatory micro-environment²⁹ and release cytokines that down-regulate the expression of several epidermal terminal differentiation markers, including filaggrin²⁰ (process h), thereby further compromising the barrier integrity¹¹. Compromised barrier integrity results in persistent inflammation even in the absence of the additional environmental stressors.

Transcription level of Gata3 is modelled by an irreversible switch⁵⁷ (Fig. 1 (C), *G-switch*): The transcription remains off ($G = G_{\text{off}}$) until sufficient numbers of DCs accumulate in the lymph

nodes to reach the activation threshold (D^+), with which the transcription initiates ($G = G_{\text{on}}$) and persists even if the DCs disappear ($D = 0$). We assume that Gata3-mediated polarization of T cells to Th2 cells is a hallmark of systemic Th2 sensitization and that the progression of AD from a mild to more severe phenotype is mediated by this irreversible G -switch.

Accordingly, our model of AD pathogenesis suggests that systemic Th2 sensitization is established if AD flares persist long enough so that $D(t)$ accumulates during the AD flares ($R = R_{\text{on}}$) and reaches D^+ to turn on the G -switch (Fig. 3 (A), right). AD flares that do not persist long enough do not turn on the G -switch, as $D(t)$ stays lower than D^+ (Fig. 3 (A), center). Our model analysis predicts that the flare time (t_{on}) longer than 2.02 days turns on the G -switch (Fig. 3(B), Eq. (E5) in the Online Repository), which is consistent with the experimental observations that the peak of Gata3 expression is reached in approximately 2 days after in vitro stimulation of Th2 cell differentiation, inducing a stable Gata3-mediated T cell differentiation³⁸. Systemic Th2 sensitization is spontaneously established in the *chronic damage* dynamic phenotype due to persistent AD flares and subsequent continuous accumulation of DCs to surpass D^+ , turning on the G -switch.

Periodic AD flares, as in the oscillation dynamic phenotype, are also predicted to turn on the G -switch, if the relaxation time (t_{off}) between AD flares (when $R = R_{\text{off}}$) is too short to prevent a gradual accumulation of DCs that eventually surpass the threshold D^+ (Fig. 3(C), right). The minimum relaxation time, above which the G -switch stays off, is analytically obtained as a function of the flare time (t_{on}) (white line in Fig. 3(D) obtained by Eq. (E6) in the Online Repository). For example, the R -spikes with $t_{\text{on}} = 1$ day turns on the G -switch if t_{off} is shorter than 0.93 days (red circle in Fig. 3(D)). Our model simulation for R -spikes with $t_{\text{on}} = 1$ day and $t_{\text{off}} = 0.9$ days predicts turning on of the G -switch within 10 days, in consistent with several features of AD skin, including Th2 cell infiltration, observed in mice after repeated application of oxazolone, once every other day over 10 days⁴⁶.

Four AD phenotypes classified by a double switch

Taken together, our model of AD pathogenesis is characterized by a double switch with two concatenated bistable switches (R - and G -switches), the status of which represents four AD phenotypes of different severity (Fig. 4(A)). The state of the R -switch determines whether the phenotype is asymptomatic ($R = R_{\text{off}}$) or symptomatic (AD flare, $R = R_{\text{on}}$), and the state of the G -switch determines whether it is systemic ($G = G_{\text{on}}$) or non-systemic ($G = G_{\text{off}}$).

For the symptomatic case, where the R -switch is constantly on and AD flares persist as in the *chronic damage* or *bistability* (unhealthy) dynamic phenotype, the non-systemic symptomatic ($R_{\text{on}}, G_{\text{off}}$)-state appears only transiently, as it inevitably progresses, after a flare time of $t_{\text{on}}=2.02$ days (Fig. 3(B)), to the ($R_{\text{on}}, G_{\text{on}}$)-state corresponding to the systemic symptomatic phenotype, the most severe AD phenotype with an impaired barrier integrity, systemic inflammation and infection.

The asymptomatic case, where the R -switch turns off after transient AD flare as in the *recovery* or *bistability* (healthy) dynamic phenotype, corresponds to either non-systemic asymptomatic (healthy) phenotype in the ($R_{\text{off}}, G_{\text{off}}$)-state or systemic asymptomatic phenotype in the ($R_{\text{off}}, G_{\text{on}}$)-state with subclinical inflammation caused by Th2-related cytokines. The systemic phenotype demonstrates more severe AD symptoms compared to its non-systemic counterpart, that are computationally characterized by a longer 95% recovery-time (Fig. 4 (B)), as suggested by experiments in both *wt* and *flg*^{-/-} mice which recovered from hapten challenge if unsensitized (non-systemic) but did not recover within two days if sensitized (systemic). The more severe symptoms are also computationally predicted by a longer treatment time required for remission (Figs. 4 (C) and 2(F)) to drive the system from the unhealthy steady state to the healthy basin of attraction for systemic than for non-systemic phenotype, due to the enlargement of the unhealthy basin of attraction. This prediction is in agreement with the clinical observation that a much

higher treatment effort is required to relieve AD symptoms during the chronic phase of AD, which is characterized by high IgE levels (systemic)¹⁷.

The more severe symptoms for the systemic, compared to the non-systemic, phenotype are also computationally predicted for the case where the R is switching between R_{off} and R_{on} as in the *oscillation* dynamic phenotype, by a decrease in the mean and an increase in the variance of the steady state of the barrier integrity (Fig. 4 (D)). This prediction is consistent with the increase in the mean and variance of the transepidermal water loss (an indicator of the barrier defect) measured in *ft* mice after the induction of systemic Th2 sensitization²¹.

Genetic risk factors predispose to systemic Th2 sensitization

Here we use our mathematical model of AD pathogenesis to investigate the synergistic effects of environmental and genetic risk factors on AD progression. Specifically, we calculate the minimum stress load (the strength of the environmental stressors, P_{env}) that can turn on the G -switch by its continuous challenge, via an AD flare of a single-pulse (Fig. 5 (A) left) or of transient oscillation (Fig. 5 (A) right), for different dynamic phenotypes caused by synergetic effects of the genetic risk factors (Fig. 2(A, B)). Turning on of the G -switch (systemic Th2 sensitization) may appear as a dramatic increase in the severity of AD symptoms as observed in *ft* and *flg*^{-/-} mice upon OVA challenges^{21,23}. The minimum stress load was experimentally evaluated in the dose-response in *wt* mice, where a systemic AD phenotype was developed by challenges with high (10%) but not lower ($\leq 2\%$) concentrations of *S. aureus* in culture supernatant⁴⁵, and by 10 repeated, but not single, oxazolone challenges⁴⁶.

Our model simulations predict that the minimum stress load is significantly lower for the *bistability* or *oscillation* dynamic phenotypes that arise in the context of genetic risk factors, compared to the *recovery* dynamic phenotype which is mainly observed when the intrinsic

genetic defects do not exist (Fig. 5 (B)). This model prediction suggests that the genetic risk factors make the patients susceptible to smaller environmental stressors in developing systemic Th2 sensitization, and is concordant with the experimental observation²² that a low concentration (0.02%) of Ox induced allergic contact dermatitis in *fl* mice, but not in *wt* mice, while higher concentration (0.5%) of Ox induced allergic contact dermatitis also in *wt* mice.

Our predicted increase in the susceptibility to environmental stressors may explain the spontaneous appearance of AD symptoms in some, but not all, carriers of genetic risk factors for AD, as demonstrated in *FLG*^{-/-} patient cohorts⁸. The carriers of the genetic risk factors who initially appear asymptomatic (as in the *bistability* or *oscillation* dynamic phenotypes, Fig. 2(C)) can demonstrate a sudden and sharp worsening of the AD symptoms when they develop systemic Th2 sensitization even with naturally occurring (small) fluctuations in the environment (Fig. 5 (B)). This observation also implies a possible mechanism behind the dose-dependent effects of filaggrin deficiency on AD symptoms⁹, as the *G*-switch is turned on by a smaller environmental load for stronger filaggrin deficiency (Fig. 5(C)).

To test the model-predicted outcome that small, naturally occurring environmental fluctuations can trigger a sudden and sharp worsening of the AD symptoms via systemic Th2 sensitization in carriers of the genetic risk factors, we evaluated the temporal changes of AD symptoms for *Stat3*-ko mice in the natural environment with small environmental stressors. Half of the mice developed an AD phenotype after 30 weeks, while the others remained asymptomatic. Mice that demonstrated an AD phenotype exhibited a much higher expression of the NF-κB-target genes (a marker of ubiquitous environmental triggers⁵⁵) (Fig. 5 (D)), suggesting that naturally occurring environmental triggers contributed to the observed development of the AD phenotype. Together with the observations in *Stat-3* ko mice that a long lasting exposure to OVA (environmental stressor) triggered a sharp increase in the levels of IgE (see Fig. E2 in the Online Repository) and that AD symptoms sharply increased after allergic systemic Th2 sensitization (increase of IgE)

(Fig. 5 (E)), these experimental results suggest that naturally occurring fluctuations in the environmental stressors can trigger systemic Th2 sensitization in mice with a genetically defective background, and worsen their AD symptoms, consistent with the model predicted outcome.

Prevention of AD symptoms by emollient treatments

We further derived the minimum stress load that can turn on the G-switch in the presence of continuous application of emollients computationally. The model predictions confirm that emollient treatments, which strengthen barrier integrity and resistance⁵⁸, reduce the susceptibility to environmental stressors in developing systemic Th2 sensitization in all asymptomatic patient cohorts considered, as demonstrated by a more than two-fold increase in the minimum stress load required to turn on the G-switch (Fig. 6). These computational results suggest that topical applications of emollients can counteract the effects of the two genetic risk factors investigated in this paper (weak barrier integrity and dysregulated immune responses) that asymptotically increase the susceptibility to environmental stressors, and effectively decrease the propensity to develop systemic Th2 sensitization in response to naturally occurring fluctuations in environment.

Importantly, our model analysis suggests preventive effects of emollients against the progression of AD, via a dynamic interplay between barrier integrity and immune reactions, for diverse patient cohorts with different genetic risk factors, not only for those with weak barrier integrity. This prediction is concordant with the results of clinical trials that demonstrated the effective prevention of the development of AD in newborns, both with and without filaggrin mutations^{26,27}, and recently observed preventive effects of emollients in AD mouse models with weak

immune responses caused by Jak-1 hyper-activation, where the incidence of AD symptoms was dramatically reduced by regular application of petrolatum on their skin⁵⁹.

Discussion

This paper proposes the first mathematical model of AD pathogenesis that reproduces gradual progression from a mostly asymptomatic mild phenotype at its early stage to a severe treatment-resistant form. Our model is a mechanistic representation of the main feedback control structure that regulates the dynamics of AD flare via a relative strength of barrier function and immune responses, corresponding to positive and negative regulation (Fig. 1).

We computationally demonstrated that the strengths of the two well-known risk factors, dysregulated immune responses and high skin barrier permeability, determine the dynamic phenotypes of AD flare by the *R*-switch, and that patients with either of the genetic risk factors may appear asymptomatic initially (Fig. 2). Long-lasting or frequent AD flares trigger the activation of the *G*-switch, which underlies the onset of systemic Th2 sensitization (Fig. 3), a hallmark event for the progression from mild to clinically severe AD (Fig. 4). Our model simulations suggested that the genetic risk factors predispose the carriers to develop a severe AD phenotype even in response to naturally occurring fluctuations in environmental stressors (Fig. 5). These susceptible patient cohorts could benefit from preventive emollient treatments, which increase the threshold stress load that drives systemic Th2 sensitization (Fig. 6).

Our model analysis identified a double switch as a key regulatory motif that determines AD phenotypes of different severity (Fig. 4). The first switch (*R*-switch) is responsible for the onset of reversible AD flare, and frequent or long-lasting AD flares can trigger the second switch (*G*-switch) leading to the irreversible progression to severe AD via systemic Th2 sensitization (Fig. 7). Description of the AD phenotypes by the status of the *R*- and *G*-switches in our model allows

us to uncover and quantitatively investigate the dynamic mechanisms behind worsening or improvement of AD symptoms, that are often evaluated clinically by an AD score (SCORAD) associated with an increased IgE and pro-inflammatory cytokines⁶⁰. The synergetic effects of environmental and genetic risk factors of AD progression determine the dynamics of the *R*- and *G*-switches that govern a dramatic worsening of AD symptoms via the onset of systemic Th2 sensitization (*G*-switch on), and could be a target for effective treatment to prevent the progression to a severe form of AD.

Our model simulations reproduced several sets of experimental and clinical results, providing plausible mechanistic, quantitative and coherent explanations for dynamic mechanisms behind onset, progression and prevention of AD. This experimentally-validated, quantitative, systems-level mathematical framework can be used to uncover possible early biomarkers and to investigate new and better treatment options to reverse and control AD symptoms and prevent progression from mild to clinically severe forms of AD. For example, application of model predictive control⁶¹ to our model will allow us to computationally design optimal treatment regimens, with minimal treatment times and strengths for effective proactive treatment^{5,56}, while reducing the risk of the side effects such as skin atrophy and barrier damage^{62,63}. Our model could also be used to investigate early warning signals⁶⁴ to identify asymptomatic but high-risk patient cohorts by their characteristic dynamics in the barrier integrity, such as higher variability (Fig. 2 (C)) or a slower recovery (Fig. 2 (E)), as they may benefit from early treatments that prevent an incipient disease development. Another promising future research includes application of machine learning methods⁶⁵ to a large set of clinical data with multiple variables to predict the likelihood of onset, progression and prevention of AD. It would be also worth evaluating the stochastic effects of the genetic variants when such data becomes available.

Consolidating effective treatment strategies for AD will potentially reduce the social and economic burden of this disease, by decreasing clinical symptoms and undesired side effects of the treatments that are associated with the advanced stage of AD. The proposed “double switch” motif provided a coherent explanation of underlying mechanisms behind dynamic onset, progression and prevention of AD. The same motif may be found and reveal similar mechanisms underlying the onset, progression and prevention of other multi-stage and multi-factorial diseases, such as cancers, diabetes, and cardiovascular disease that are caused by a complex interplay between genetic and environmental factors.

Methods

Model description

The proposed model of AD pathogenesis (Fig. 1) is described by a hybrid system,

$$\frac{dP(t)}{dt} = P_{\text{env}} \frac{\kappa_P}{1 + \gamma_B B(t)} - \alpha_I R(t)P(t) - \delta_P P(t), \quad (1a)$$

$$\frac{dB(t)}{dt} = \kappa_B \frac{1}{1 + \gamma_R R(t)} \frac{1}{1 + \gamma_G G(t)} (1 - B(t)) - \delta_B K(t)B(t), \quad (1b)$$

$$\frac{dD(t)}{dt} = \kappa_D R(t) - \delta_D D(t), \quad (1c)$$

for the dynamics of the three variables, $P(t)$, $B(t)$ and $D(t)$, denoting the infiltrated pathogen load (mg/ml), the strength of barrier integrity (relative to the maximum strength), and the concentration of DCs in the lymph node (cells/ml), respectively. The dynamics of $P(t)$, $B(t)$ and $D(t)$ depend on the dynamics of the three additional variables, $R(t)$, $G(t)$ and $K(t)$, denoting the levels of activated immune receptors, Gata3 transcription (relative to the maximum transcription level), and active kallikreins, respectively.

The infiltrated pathogen load, P , increases by the penetration of environmental stress load, P_{env} , through the barrier, B . P is eradicated by innate immune responses triggered by inflammation (R) and is also naturally degraded. The barrier function, B , is determined by the balance between its production and degradation. The barrier production is described by a phenomenological representation of its capacity to self-restore the nominal barrier function following its disruption, and is compromised by innate immune responses triggered by inflammation (R) and cytokines produced by differentiated Th2 cells which increases according to G . The degradation of the barrier occurs as a result of desquamation mediated by active kallikreins, K . The DCs, D , increases while inflammation (R) persists and degrades naturally. The equations (1) use simple mathematical terms based on the law of mass action with zero order production and first order degradation terms, except for the barrier production term, $\kappa_x(1 - x(t))$, which represents the phenomenological recovery of skin barrier in response to disruption⁶⁶. The inhibitory rates are described in the form of $1/(1 + x)$.

The dynamics of $R(t)$, $G(t)$ and $K(t)$ is described by a perfect switch (Fig. 1(C)),

$$(R(t), K(t)) = \begin{cases} (R_{\text{off}}, K_{\text{off}}) & \text{if } P(t) < P^- \text{ or } \{P^- \leq P(t) \leq P^+ \text{ and } R(t^-) = R_{\text{off}}\} \\ (R_{\text{on}}, K_{\text{on}} = m_{\text{on}}P(t) - \beta) & \text{if } P(t) > P^+ \text{ or } \{P^- \leq P(t) \leq P^+ \text{ and } R(t^-) = R_{\text{on}}\} \end{cases} \quad (2)$$

$$G(t) = \begin{cases} G_{\text{off}} & \text{if } D(t) < D^+ \text{ and } G(t^-) = G_{\text{off}} \\ G_{\text{on}} & \text{if } D(t) \geq D^+ \text{ or } G(t^-) = G_{\text{on}} \end{cases} \quad (3)$$

where t^- is a time slightly before the time t , as they change abruptly within hours^{47,67}, in a much faster time-scale than for $P(t)$, $B(t)$ and $D(t)$ that change over weeks^{66,68}.

The nominal values of model parameters (Table I) were taken from the previously published model of the early stage of AD²⁴, obtained by approximation of the bifurcation diagrams previously derived^{50,57}, or derived from experimental literature^{20,66,68-70}. Emollient treatments are modelled by a constant increase in the barrier production rate ($dB/dt=1.5$).

Model analysis

Model analysis was conducted using MATLAB version R2014a (The MathWorks, Inc., Natick, MA, USA). We used *ode15s* for numerical integration of the system from the steady states as the initial conditions, and *output* function to update the switching variables at each step of the iteration. Our model analysis investigated the ranges of $0 \leq \alpha_i \leq 0.3$ and $0 \leq \kappa_p \leq 1$. The dynamic trajectories were calculated for 1054 pairs of (α_i, κ_p) uniformly sampled (an interval of 0.01 for α_i and 0.03 for κ_p). The bifurcation diagram (Fig. 2(A)) of the proposed model was numerically obtained using the methodology described in ⁷¹ to identify the four qualitatively different dynamic phenotypes with respect to the relative strengths of the feedbacks determined by skin barrier permeability (κ_p) and immune responses (α_i). The circles in Fig. 2(A) represent the parameter values used to generate the example dynamics for each of these dynamic phenotypes (Fig. 2(B)). The steady states for the barrier integrity (Figs. 2(C, D) and 4(D)) were calculated analytically for the *recovery*, *bistability* and *chronic damage* dynamic phenotypes, and numerically obtained for the *oscillation* dynamic phenotype by taking the mean of the mean-over-time over the corresponding parameter region for (α_i, κ_p) for Fig. 2(C) and by taking the mean for varied levels of skin barrier permeability (κ_p) with $\alpha_i = 0.25$ for Fig. 2(D). The basins of attraction for the *bistability* dynamic phenotype (Figs. 2(F) and 4(C) for $(\alpha_i, \kappa_p) = (0.04, 0.65)$) were numerically determined by integrating the system from the points of tangency, (P^-, B^-) and (P^+, B^+) , identified by solving $\dot{P}(t) = 0$ at $P(t) = P^-$ and P^+ , respectively. In Fig. 2(F), the healthy and unhealthy steady states are represented by black and red filled circles, and their basins of attraction by the grey and red regions. The states in the yellow region converge to the healthy steady state if the initial condition is $R = R_{\text{off}}$ and to the unhealthy steady state if $R = R_{\text{on}}$. The minimal stress load that can trigger systemic Th2 sensitization (Figs. 5(B, C) and 6(A-C)) were numerically determined by evaluating whether the $D(t)$ surpasses the threshold, D^+ . The 95% recovery times (Figs. 2 (E) and 4 (B)) were numerically obtained by $t_{95\%} - t_{\text{min}}$, where $B(t_{95\%}) = 0.95$ and $B(t_{\text{min}})$ is the minimum barrier integrity achieved after a transient AD flare. The boxplots in Figs. 2(C, E), 4(B, C, D), 5(B, E) and 6 show the minimum, first quartile, median, third quartile, and maximum values.

Epidermis-specific Stat3-ko mice

We developed keratinocyte-specific *Stat3*-ko (*Stat3^{f/f}*) mice with B6X129 mixed background⁴². All mice used in this study were maintained under SPF conditions. Animal care was conducted in accordance with guidelines of the RIKEN Yokohama Institute.

Development of AD phenotypes in epidermis-specific *Stat3*-ko mice

Expression levels of 11 NF- κ B-target genes (*Bcl2*, *Ddx26b*, *Gadd45b*, *Icam1*, *Icam2*, *Icam4*, *Icam5*, *Il1b*, *Tnf*, *Traf1*, *Traf2* and *Vcam1*) selected from the KEGG database (http://www.genome.jp/kegg-bin/show_pathway?ko04064+K02580) were quantified from sorted ear samples of 2, 5, 8 and 10 weeks old *Stat3^{f/f}* mice. Using total RNA isolated with TRIzol, cDNAs were synthesized with NEBNext Ultra RNA Library Prep Kit for Illumina (NEB Biolabs, Inc., Ipswich, MA) according to the manufacturer's instructions. Comprehensive expression analysis was performed by mRNA sequence (single-ended 50-base pair reads) using the HiSeq 3000 system (Illumina) (GEO accession number GSE86071). Read alignment (mm9) was performed using TopHat2 v2.0.8 and expression level was determined by Cufflinks v2.1.1 with default settings. We calculated the mean expression levels of the NF κ B-target genes over the $n = 2$ mice per cohort (asymptomatic and AD phenotype) and normalized them by the wt dynamics (Fig. 5(D)).

Severity of the AD symptoms (Fig. 5(E)) was assessed in $n = 11$ mice at 51 time points, by examining the areas of the skin lesion: no lesion (score 0), periocular lesions (score 1/5), lesions in half side of the face (score 2/5), in a complete side of the face (score 3/5), in the whole face (score 4/5), or in the whole face and the ears (score 5/5). The time of systemic sensitization ($t = 0$) corresponds to the time when the IgE levels rose to 10% of the maximal IgE level measured for each mouse.

Figure legends

Figure 1: Mechanistic model of AD pathogenesis with a double switch motif. (A) Schematic diagram of the processes included in the model. Italics denote the state variables in the model equations (Eq. (1)). (B) Control structure of the system regulating AD flares by positive and negative feedbacks. (C) *R*-switch (reversible activation of innate immune receptors) and *G*-switch (irreversible Gata3 transcription).

Figure 2: Computationally predicted effects of the two main genetic risk factors on the dynamic response to environmental stressors. (A) A schematic of the bifurcation diagram. (B) Four dynamic phenotypes. (C) Steady states of barrier integrity. (D) Dose-dependency of filaggrin deficiency. (E) Time required to regain 95% of the healthy steady state of barrier integrity. (F) Basin of attraction in the (P , B)-space.

Figure 3: Proposed mechanisms of AD progression via double switch. The *G*-switch is turned on by accumulation of DCs beyond D^+ , due to transient AD flares (A) with a flare time (t_{on}) longer than 2.02 days (B), or periodic AD flares (C) with a sufficiently small relaxation time (t_{off}) as a function of t_{on} (D).

Figure 4: Four AD phenotypes described by the status of the double switch. (A) The *R*-switch distinguishes between asymptomatic ($R=R_{off}$) and symptomatic ($R=R_{on}$), and the *G*-switch between non-systemic ($G=G_{off}$) and systemic ($G=G_{on}$) phenotypes. Systemic phenotypes demonstrate severe AD symptoms compared to non-systemic counterparts, as computationally predicted by (B) longer 95% recovery time, (C) longer treatment time required for remission, and (D) lower barrier integrity.

Figure 5: Genetic risk factors increase the susceptibility to environmental stressors in developing systemic Th2 sensitization. (A) Transient increases in the environmental stress load can trigger systemic Th2 sensitization. The minimum stress load decreases with genetic risk factors (B) in a dose-dependent manner (C). *Stat3*-ko mice that developed AD symptoms had higher NF- κ B activity (D), and the severity of the symptoms increased sharply after Th2 sensitization (E).

Figure 6: Emollient treatments prevent the progression to severe AD, by increasing the computationally predicted minimum stress load required to drive systemic inflammation in (A) *recovery*, (B) *bistability*, and (C) *oscillation* dynamic phenotypes, by approximately 2-fold (D).

Figure 7: "Double switch" mechanism for onset and progression of atopic dermatitis (AD) proposed in this paper. Environmental stressors may cause onset of the first reversible switch leading to AD flares, which can be reversed by treatments. Frequent or long-lasting AD flares can trigger the second irreversible switch resulting in progression to severe AD.

References

1. Weidinger S, Novak N. Atopic dermatitis. *Lancet* 2016;387:1109–22.
2. Bergman JN, Cohen DE, Cooper KD, Cordero KM, Davis DM, Krol A, et al. Guidelines of care for the management of atopic dermatitis. *J Am Acad Dermatol* 2014;70:338–51.
3. Spergel JM. Epidemiology of atopic dermatitis and atopic march in children. *Immunol Allergy Clin North Am* 2010 [cited 2016 Feb 13];30:269–80.
4. Bieber T, Akdis C, Lauener R, Traidl-Hoffmann C, Schmid-Grendelmeier P, Schäppi G, et al. Global Allergy Forum and 3rd Davos Declaration 2015: Atopic dermatitis/Eczema: Challenges and opportunities toward precision medicine Global Allergy Forum and 3rd Davos Declaration 2015: Atopic dermatitis/Eczema: Challenges and opportunities toward precis. *Allergy Eur J Allergy Clin Immunol* 2016;71:588–92.
5. Wollenberg A, Ehmann LM. Long Term Treatment Concepts and Proactive Therapy for Atopic Eczema. *Ann Dermatol* 2012;24:253–60.
6. Tanaka RJ, Ono M. Skin Disease Modeling from a Mathematical Perspective. *J Invest Dermatol* 2013;133:1472–8.
7. Reynolds NJ. One hundred and twenty-five years and counting: into an era of systems dermatology. *Br J Dermatol* 2014;171:1279–81.
8. Palmer CN a, Irvine AD, Terron-Kwiatkowski A, Zhao Y, Liao H, Lee SP, et al. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet* 2006;38:441–6.
9. Brown SJ, Kroboth K, Sandilands A, Campbell LE, Pohler E, Kezic S, et al. Intragenic copy number variation within filaggrin contributes to the risk of atopic dermatitis with a dose-dependent effect. *J Invest Dermatol* 2012;132:98–104.
10. Irvine AD, McLean WHI, Leung DYM. Filaggrin Mutations Associated with Skin and Allergic Diseases. *N Engl J Med* 2011;365:1315–27.
11. Nemoto-Hasebe I, Akiyama M, Nomura T, Sandilands A, McLean WHI, Shimizu H. Clinical severity correlates with impaired barrier in filaggrin-related eczema. *J Invest Dermatol* 2009;129:682–9.
12. Paternoster L, Standl M, Waage J, Baurecht H, Hotze M, Strachan DP, et al. Multi-ancestry genome-wide association study of 21,000 cases and 95,000 controls identifies new risk loci for atopic dermatitis. *Nat Genet* 2015;47:1449–56.
13. McGirt LY, Beck L A. Innate immune defects in atopic dermatitis. *J Allergy Clin Immunol* 2006;118:202–8.
14. Kezic S, O'Regan GM, Lutter R, Jakasa I, Koster ES, Saunders S, et al. Filaggrin loss-of-function mutations are associated with enhanced expression of IL-1 cytokines in the stratum corneum of patients with atopic dermatitis and in a murine model of filaggrin deficiency. *J Allergy Clin Immunol* 2012;129:1031–9.e1.
15. Kong HH, Oh J, Deming C, Conlan S, Grice E A, Beatson M, et al. Temporal shifts in the skin microbiome associated with atopic dermatitis disease flares and treatment. *Genome Res* 2012;850–9.
16. Williams MR, Gallo RL. The Role of the Skin Microbiome in Atopic Dermatitis. *Curr Allergy Asthma Rep* 2015;15:65.
17. Taïeb A, Seneschal J, Mossalayi MD. Biologics in atopic dermatitis. *J Dtsch Dermatol Ges* 2012;10:174–8.
18. McAleer M a, Irvine AD. The multifunctional role of filaggrin in allergic skin disease. *J Allergy Clin Immunol* 2013;131:280–91.
19. Cork MJ, Robinson D A, Vasilopoulos Y, Ferguson A, Moustafa M, Macgowan AL, et al. New perspectives on epidermal barrier dysfunction in atopic dermatitis: gene-environment interactions. *J Allergy Clin Immunol* 2006;118:3–21;
20. Howell MD, Kim BE, Gao P, Grant A V, Boguniewicz M, Debenedetto A, et al. Cytokine modulation of atopic dermatitis filaggrin skin expression. *J Allergy Clin Immunol* 2007;120:150–5.
21. Fallon PG, Sasaki T, Sandilands A, Campbell LE, Saunders SP, Mangan NE, et al. A homozygous frameshift mutation in the mouse Flg gene facilitates enhanced percutaneous allergen priming. *Nat Genet* 2009;41:602–8.
22. Scharschmidt TC, Man M-Q, Hatano Y, Crumrine D, Gunathilake R, Sundberg JP, et al. Filaggrin deficiency confers a paracellular barrier abnormality that reduces inflammatory thresholds to irritants and haptens. *J Allergy Clin Immunol* 2009;124:496–506, 506.e1–6.
23. Kawasaki H, Nagao K, Kubo A, Hata T, Shimizu A, Mizuno H, et al. Altered stratum corneum barrier and enhanced percutaneous immune responses in filaggrin-null mice. *J Allergy Clin Immunol* 2012;129:1538–46.

24. Domínguez-Hüttner E, Ono M, Barahona M, Tanaka RJ. Risk factor-dependent dynamics of atopic dermatitis: modelling multi-scale regulation of epithelium homeostasis. *Interface Focus* 2013;3:20120090.
25. Elias PM, Wakefield JS. Mechanisms of abnormal lamellar body secretion and the dysfunctional skin barrier in patients with atopic dermatitis. *J Allergy Clin Immunol* 2014;134:781–91.e1.
26. Simpson EL, Chalmers JR, Hanifin JM, Thomas KS, Cork MJ, Mclean WHI, et al. Emollient enhancement of the skin barrier from birth offers effective atopic dermatitis prevention. *J Allergy Clin Immunol* 2014;134:818–23.
27. Horimukai K, Morita K, Narita M, Kondo M, Kitazawa H. Application of moisturizer to neonates prevents development of atopic dermatitis. *J Allergy Clin Immunol* 2014;134:824–30.e6.
28. Jinnestål CL, Belfrage E, Bäck O, Schmidtchen A, Sonesson A. Skin barrier impairment correlates with cutaneous *Staphylococcus aureus* colonization and sensitization to skin-associated microbial antigens in adult patients with atopic dermatitis. *Int J Dermatol* 2014;53:27–33.
29. Gittler JK, Shemer A, Suárez-Fariñas M, Fuentes-Duculan J, Gulewicz KJ, Wang CQF, et al. Progressive activation of T(H)2/T(H)22 cytokines and selective epidermal proteins characterizes acute and chronic atopic dermatitis. *J Allergy Clin Immunol* 2012;130:1344–54.
30. Galli SJ, Tsai M, Piliponsky AM. The development of allergic inflammation. *Nature* 2008;454:445–54.
31. Hachem J-P, Crumrine D, Fluhr JW, Brown BE, Feingold KR, Elias PM. pH directly regulates epidermal permeability barrier homeostasis, and stratum corneum integrity/cohesion. *J Invest Dermatol* 2003;121:345–53.
32. Aberg KM, Man M-Q, Gallo RL, Ganz T, Crumrine D, Brown BE, et al. Co-regulation and interdependence of the mammalian epidermal permeability and antimicrobial barriers. *J Invest Dermatol* 2008;128:917–25.
33. Cenac N, Chin AC, Garcia-Villar R, Salvador-Cartier C, Ferrier L, Vergnolle N, et al. PAR2 activation alters colonic paracellular permeability in mice via IFN-gamma-dependent and -independent pathways. *J Physiol* 2004;558:913–25.
34. Briot A, Deraison C, Lacroix M, Bonnart C, Robin A, Besson C, et al. Kallikrein 5 induces atopic dermatitis-like lesions through PAR2-mediated thymic stromal lymphopoietin expression in Netherton syndrome. *J Exp Med* 2009;206:1135–47.
35. Das J, Chen C, Yang L, Cohn L, Ray P, Ray A. A critical role for NF- κ B in Gata3 expression and TH 2 differentiation in allergic airway inflammation. *Nature* 2001;2.
36. Duckney P, Wong HK, Serrano J, Yaradou D, Oddos T, Stamatas GN. The role of the skin barrier in modulating the effects of common skin microbial species on the inflammation, differentiation and proliferation status of epidermal keratinocytes. *BMC Res Notes* 2013;6:474.
37. Yamasaki K, Kanada K, Macleod DT, Borkowski AW, Morizane S, Nakatsuji T, et al. TLR2 expression is increased in rosacea and stimulates enhanced serine protease production by keratinocytes. *J Invest Dermatol* 2011;131:688–97.
38. Zheng W, Flavell R. The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. *Cell* 1997;89:587–96.
39. Leung DYM, Guttman-Yassky E. Deciphering the complexities of atopic dermatitis: Shifting paradigms in treatment approaches. *J Allergy Clin Immunol* 2015;134:769–79.
40. Sasaki T, Shiohama A, Kubo A, Kawasaki H. 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* 2013;132:1111–20.e4.
41. Saunders SP, Goh CSM, Brown SJ, Palmer CN a, Porter RM, Cole C, et al. Tmem79/Matt is the matted mouse gene and is a predisposing gene for atopic dermatitis in human subjects. *J Allergy Clin Immunol* 2013;132:1121–9.
42. Sano S, Itami S, Takeda K, Tarutani M, Yamaguchi Y, Miura H, et al. Keratinocyte-specific ablation of Stat3 exhibits impaired skin remodeling, but does not affect skin morphogenesis. *EMBO J* 1999;18:4657–68.
43. Hsu AP, Uzel G, Brodsky N, Freeman AF, Demidowich A, Davis J, et al. Mutations in the Hyper-IgE Syndrome. 2007;1608–19.
44. Okuma A, Hoshino K, Ohba T, Fukushima S, Aiba S, Akira S, et al. Enhanced Apoptosis by Disruption of the STAT3-I κ B- ζ Signaling Pathway in Epithelial Cells Induces Sjögren's Syndrome-like Autoimmune Disease. *Immunity* 2013;38:450–60.
45. Nakamura Y, Oscherwitz J, Cease KB, Chan SM, Muñoz-Planillo R, Hasegawa M, et al. *Staphylococcus* δ -toxin induces allergic skin disease by activating mast cells. *Nature* 2013;503:397–401.
46. Man M-Q, Hatano Y, Lee SH, Man M, Chang S, Feingold KR, et al. Characterization of a hapten-induced, murine model with multiple features of atopic dermatitis: structural, immunologic, and biochemical changes following single versus multiple oxazolone challenges. *J Invest Dermatol* 2008;128:79–86.

47. Dommisch H, Chung WO, Rohani MG, Williams D, Rangarajan M, Curtis M a., et al. Protease-activated receptor 2 mediates human beta-defensin 2 and CC chemokine ligand 20 mRNA expression in response to proteases secreted by *Porphyromonas gingivalis*. *Infect Immun* 2007;75:4326–33.
48. Imai Y, Yasuda K, Sakaguchi Y, Haneda T, Mizutani H, Yoshimoto T, et al. Skin-specific expression of IL-33 activates group 2 innate lymphoid cells and elicits atopic dermatitis-like inflammation in mice. *Proc Natl Acad Sci U S A* 2013;110:13921–6.
49. Hams E, Bermingham R, Fallon PG. Macrophage and innate lymphoid cell interplay in the genesis of fibrosis. *Front Immunol* 2015;6:1–11.
50. Tanaka RJ, Ono M, Harrington H a. Skin barrier homeostasis in atopic dermatitis: feedback regulation of kallikrein activity. *PLoS One* 2011;6:e19895.
51. Ahmad-Nejad P, Mrabet-Dahbi S, Breuer K, Klotz M, Werfel T, Herz U, et al. The toll-like receptor 2 R753Q polymorphism defines a subgroup of patients with atopic dermatitis having severe phenotype. *J Allergy Clin Immunol* 2004;113:565–7.
52. Zasloff M. Antimicrobial peptides of multicellular organisms. *Nature* 2002;415:389–95.
53. Sugiura A, Nomura T, Mizuno A, Imokawa G. Reevaluation of the non-lesional dry skin in atopic dermatitis by acute barrier disruption: an abnormal permeability barrier homeostasis with defective processing to generate ceramide. *Arch Dermatol Res* 2014;306:427–40.
54. Hoffman DR, Kroll LM, Basehoar A, Reece B, Cunningham CT, Koenig DW. Immediate and extended effects of abrasion on stratum corneum natural moisturizing factor. *Ski Res Technol* 2015;1–7.
55. Akira S, Takeda K. Toll-like receptor signalling. *Nat Rev Immunol* 2004;4:499–511.
56. Tang TS, Bieber T, Williams HC. Are the concepts of induction of remission and treatment of subclinical inflammation in atopic dermatitis clinically useful? *J Allergy Clin Immunol* 2014;133:1–12.
57. Höfer T, Nathansen H, Löhning M, Radbruch A, Heinrich R. GATA-3 transcriptional imprinting in Th2 lymphocytes: a mathematical model. *PNAS* 2002;99:9364–8.
58. van Logtestijn MD., Domínguez-Hüttinger E, Stamatas GN, Tanaka RJ. Resistance to Water Diffusion in the Stratum Corneum Is Depth-Dependent. *PLoS One* 2015;10:e0117292.
59. Yasuda T, Fukada T, Nishida K, Nakayama M, Matsuda M, Miura I, et al. Hyperactivation of JAK1 tyrosine kinase induces stepwise, progressive pruritic dermatitis. *J Clin Invest* 2016;1–13.
60. Vakirlis E, Lazaridou E, Tzellos TG, Gerou S, Chatzidimitriou D, Ioannides D. Investigation of cytokine levels and their association with SCORAD index in adults with acute atopic dermatitis. *J Eur Acad Dermatology Venereol* 2011;25:409–16.
61. Hirata Y, Bernardo M, Bruchovsky N, Aihara K, Hirata Y, Bruchovsky N, et al. Hybrid optimal scheduling for intermittent androgen suppression of prostate cancer. *Chaos* 2013;045125.
62. Schoepe S, Schäcke H, May E, Asadullah K. Glucocorticoid therapy-induced skin atrophy. *Exp Dermatol* 2006;15:406–20.
63. Danby SG, Chittock J, Brown K, Albenali LH, Cork MJ. The effect of tacrolimus compared to betamethasone valerate on the skin barrier in volunteers with quiescent atopic dermatitis. *Br J Dermatol* 2014;170:914–21.
64. Chen L, Liu R, Liu Z-P, Li M, Aihara K. Detecting early-warning signals for sudden deterioration of complex diseases by dynamical network biomarkers. *Sci Rep* 2012;2:342.
65. Shouval R, Bondi O, Mishan H, Shimoni A, Unger R, Nagler A. G: a data-mining approach in SCT. *Bone Marrow Transplant* 2014;49:332–7.
66. Hoffman DR, Kroll LM, Basehoar A., Reece B, Cunningham CT, Koenig DW. Immediate and extended effects of sodium lauryl sulphate exposure on stratum corneum natural moisturizing factor. *Int J Cosmet Sci* 2014;36:93–101.
67. Yamane H, Zhu J, Paul WE. Independent roles for IL-2 and GATA-3 in stimulating naive CD4+ T cells to generate a Th2-inducing cytokine environment. *J Exp Med* 2005;202:793–804.
68. Zhang Z, Clarke TB, Weiser JN. Cellular effectors mediating Th17-dependent clearance of pneumococcal colonization in mice. *J Clin Invest* 2009;119:1899–909.
69. Chen M, Huang L, Shabier Z, Wang J. Regulation of the lifespan in dendritic cell subsets. *Mol Immunol* 2007;44:2558–65.
70. Celli S, Day M, Mu AJ, Molina-paris C, Lythe G, Bousso P. How many dendritic cells are required to initiate a T-cell response? *Blood* 2012;120:3945–8.
71. Oyarzún D, Chaves M, Hoff-Hoffmeyer-Zlotnik M. Multistability and oscillations in genetic control of metabolism. *J Theor Biol* 2012;295:139–53.

712 **Acknowledgements**

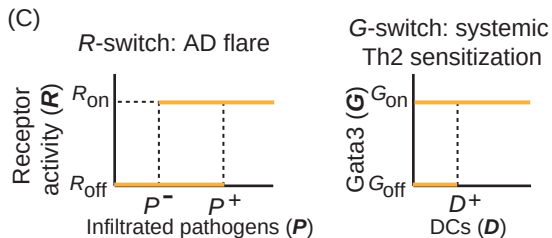
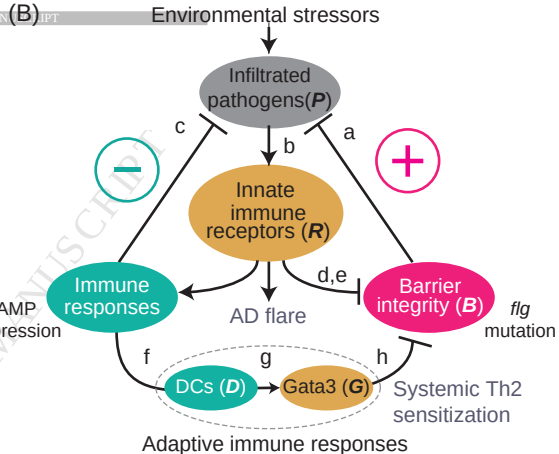
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715 mice.

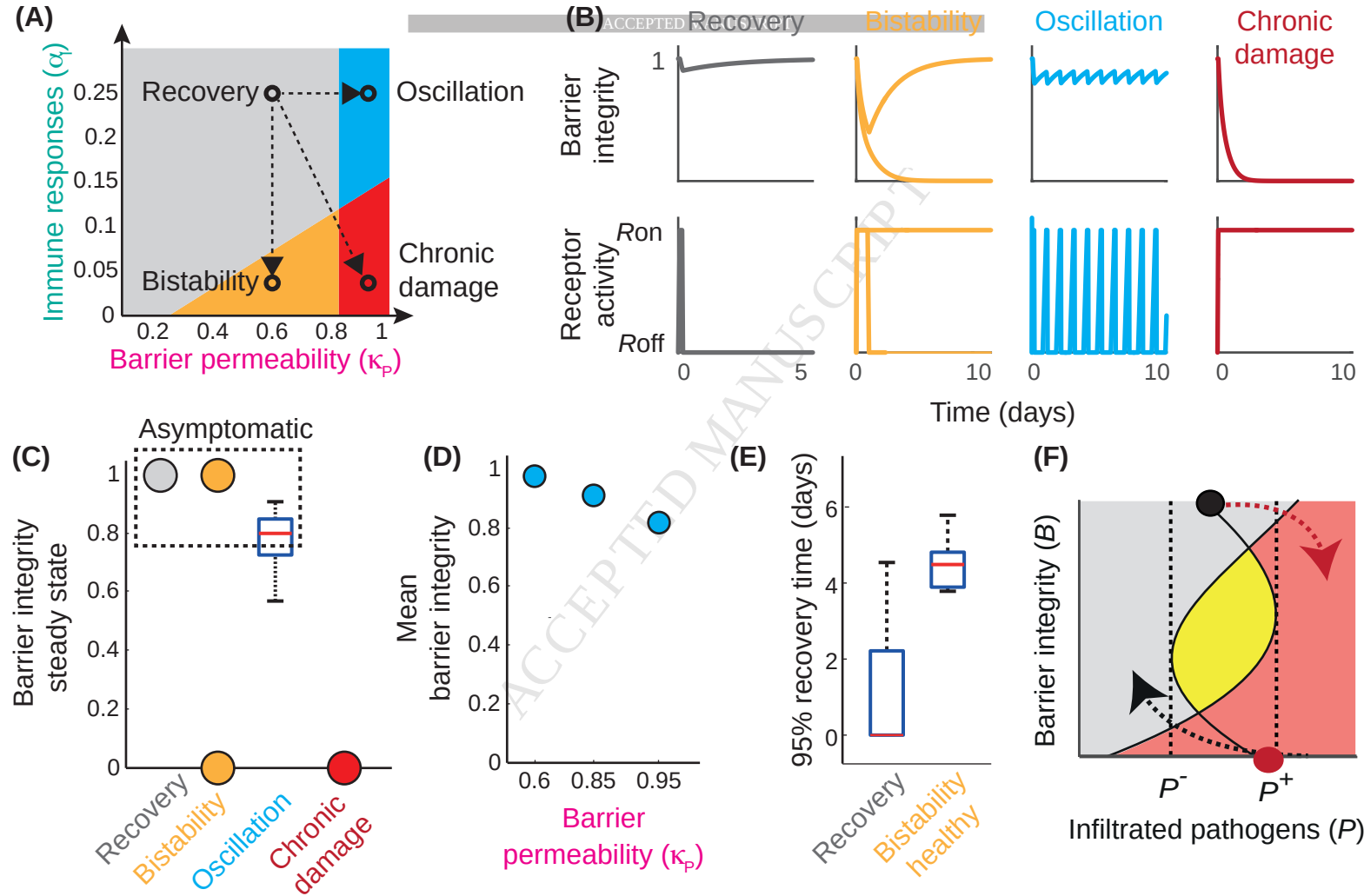
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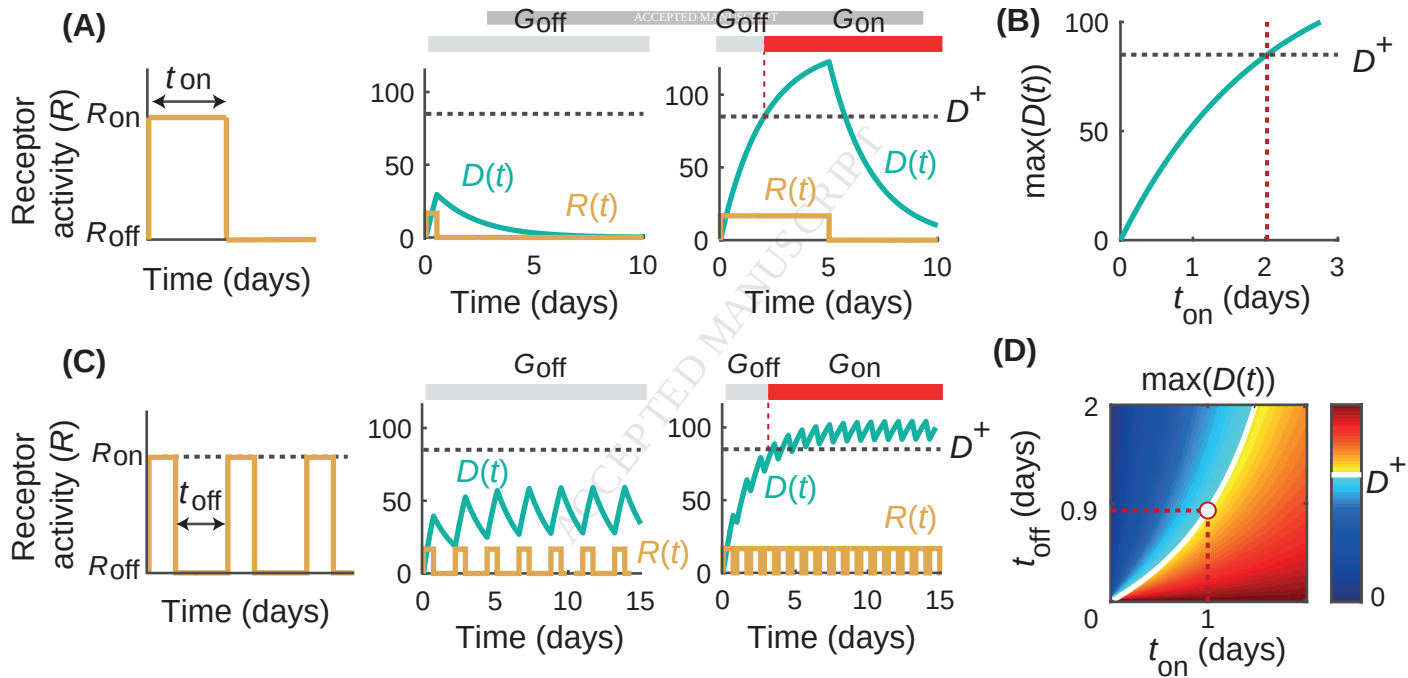
Table I: Definition of model parameters and their nominal values.

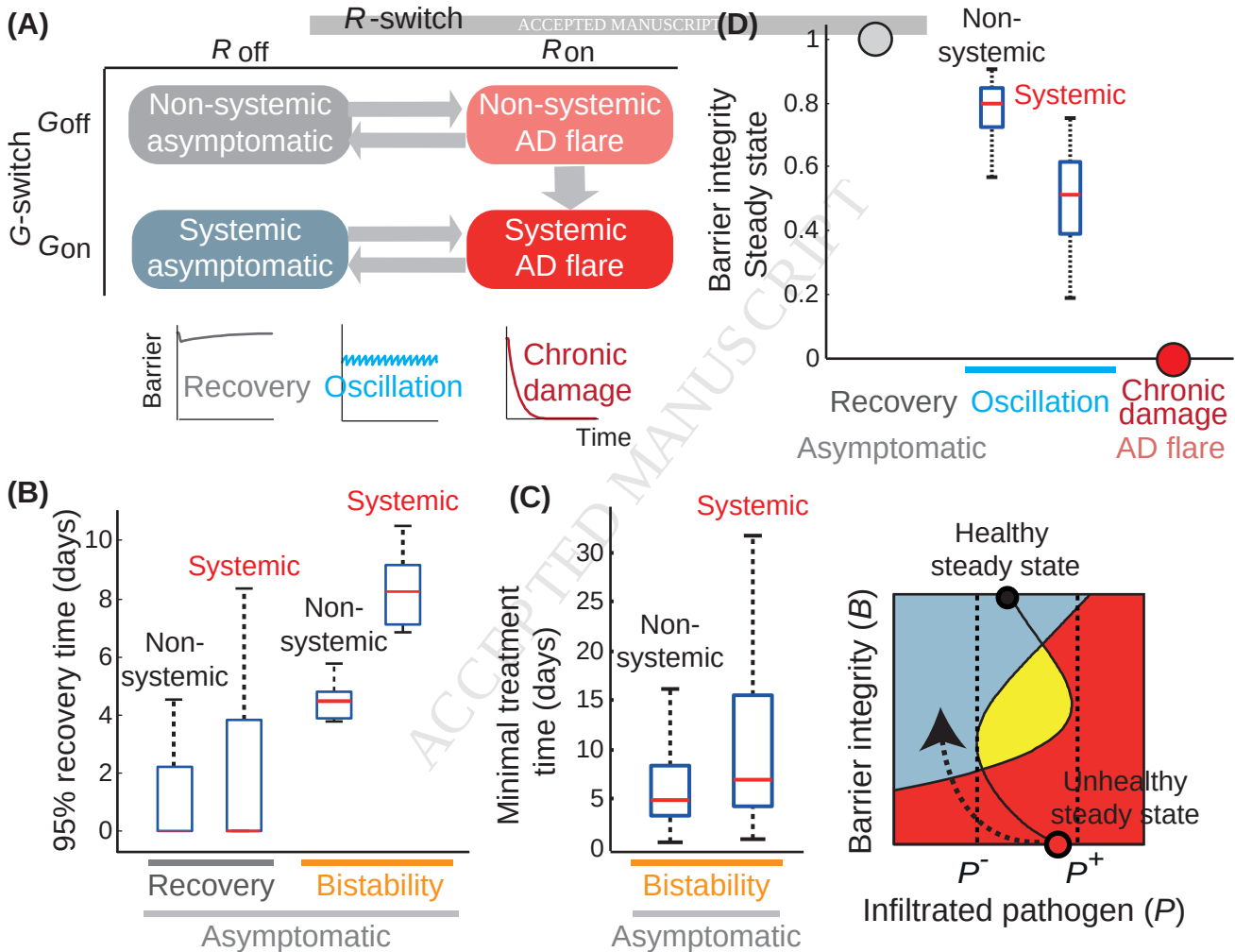
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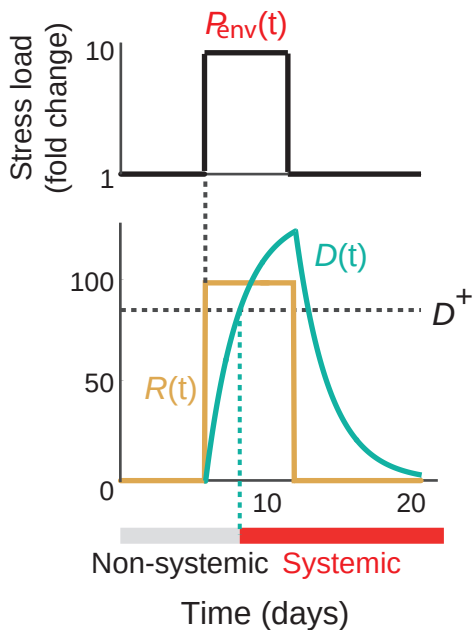
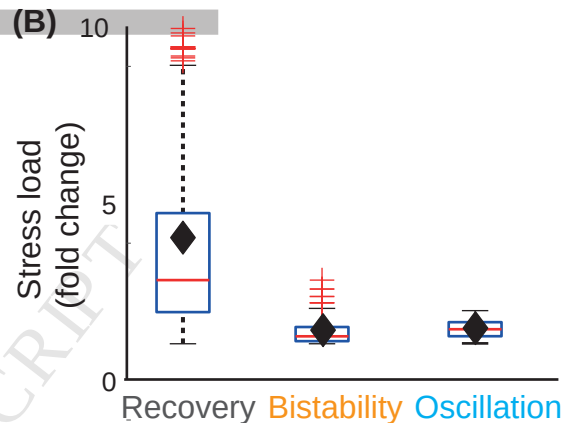
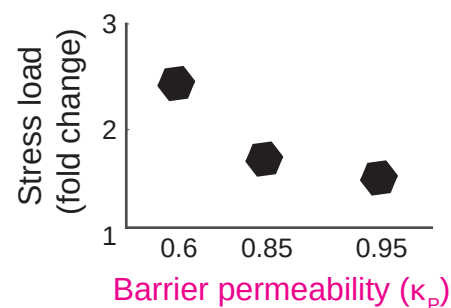
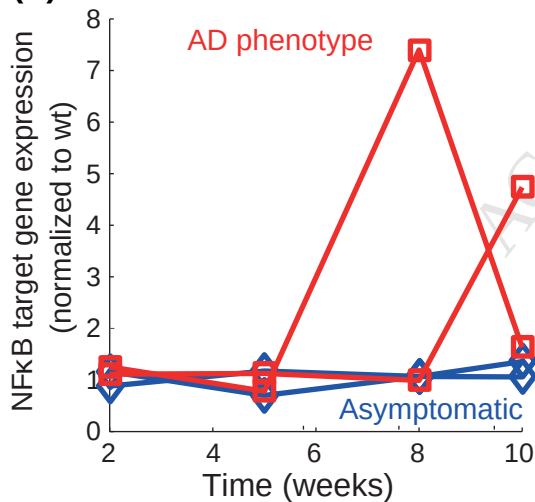
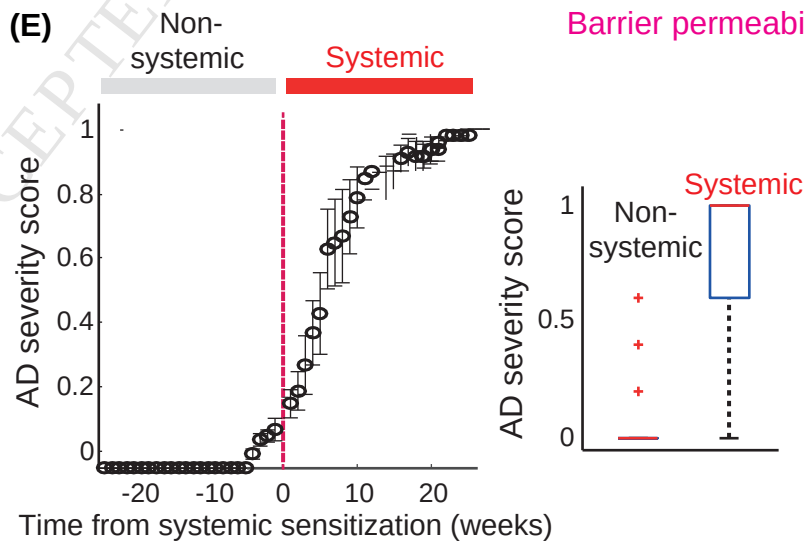
Symbol	Name	Nominal value	Ref.
P_{env}	Environmental stress load	95 (mg/ml)	[24]
γ_B	Barrier-mediated inhibition of pathogen infiltration	1	[24]
κ_P	Nominal skin permeability	0.6 (1/day)	[24]
α_I	Rate of pathogen eradication by innate immune responses	0.25 (1/day)	[24]
δ_P	Basal pathogen death rate	1 (1/day)	[24]
κ_B	Barrier production rate	0.5 (1/day)	[24, 66]
γ_R	Innate immunity-mediated inhibition of barrier production	10	[24]
δ_B	Rate of kallikrein-dependent barrier degradation	0.1	[24]
γ_G	Adaptive immunity-mediated inhibition of barrier production	1	[20]
κ_D	Rate of DC activation by receptors	4 cells/(ml $\times$ day)	[65]
δ_D	Rate of DC degradation	0.5 (1/day)	[70]
P^-	Receptor inactivation threshold	26.6 (mg/ml)	[50]
P^+	Receptor activation threshold	40 (mg/ml)	[50]
D^+	Gata3 activation threshold	85 (cells/ml)	[57, 68]
R_{off}	Receptor-off level	0	[50]
R_{on}	Receptor-on level	16.7	[50]
G_{off}	Gata3-off level	0	[57]
G_{on}	Gata3-on level	1	[57]
K_{off}	Kallikrein-off level	0	[50]
m_{on}	Slope of the linear relation between $P(t)$ and K_{on}	0.45	[50]
β_{on}	Y-intercept of the linear relation between $P(t)$ and K_{on}	6.71	[50]





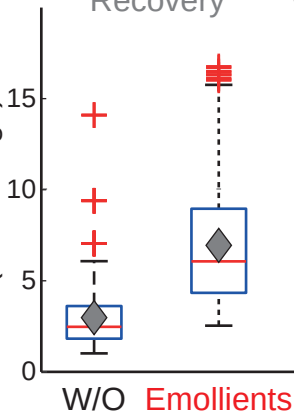




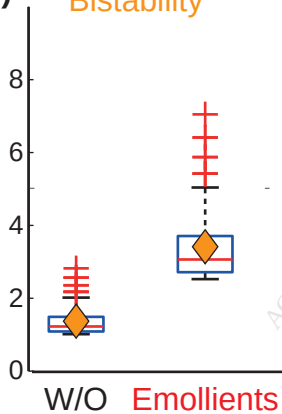
(A)**(B)****(C)****(D)****(E)**

(A)Minimal stress load
(fold change)

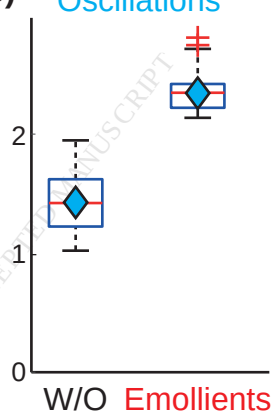
Recovery

**(B)**

Bistability

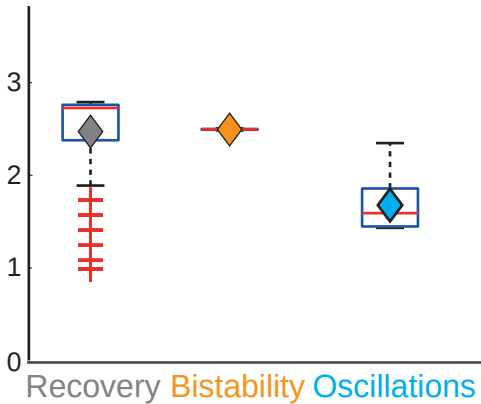
**(C)**

Oscillations

**(D)**

Ratio of minimal stress load

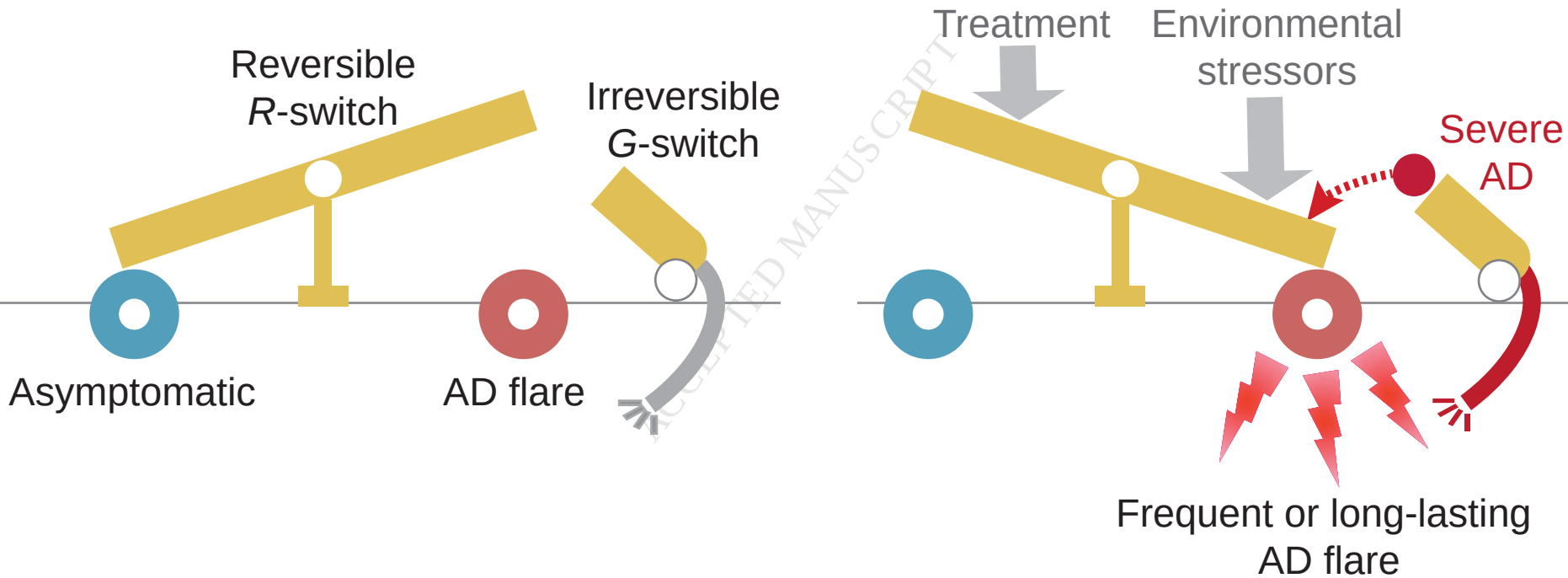
Recovery Bistability Oscillations



Asymptomatic - no inflammation

ACCEPTED MANUSCRIPT

Onset and progression of AD



Supplementary Dataset E2: Experimental data on the dynamic expression of 11 NFkB-target genes (Fig. 5(D)).

NFkB target gene	time (weeks)			
	2	5	8	10
	Control - mouse 1			
	C1_2w	C1_5w	C1_8w	C1_10w
Bcl2	1,49096	1,00037	1,60742	2,25852
Ddx26b	12,1838	12,8617	13,7913	18,9728
Gadd45b	10,2747	10,4486	24,0344	14,2288
Icam1	11,1869	8,10447	14,1105	15,6618
Icam2	5,67296	2,29207	2,83728	1,86459
Icam4	0,479092	0,0986578	0,258129	0,995197
Icam5	0,145468	0,0998525	0,566053	0,553987
Il1b	13,7828	0,55531	2,04485	0,373441
Tnf	5,23604	1,47033	3,12033	1,28542
Traf1	1,04792	0,686619	0,826949	1,31927
Traf2	7,73797	7,76872	9,09156	9,28874
Vcam1	12,4412	4,71144	4,69602	4,27077
Mean NFkB target gene expression (control mouse 1)	6,806650833	4,1748449	6,4153993	5,9227779

NFkB target gene	Control - mouse 2			
	C2_2w	C2_5w	C2_8w	C2_10w
Bcl2	1,46856	2,09932	1,73659	1,02413
Ddx26b	12,834	20,853	17,0093	13,4294
Gadd45b	15,0294	16,3331	14,8715	8,53494
Icam1	9,57862	16,4514	11,0136	8,20556
Icam2	5,50534	2,59863	3,39916	2,74231
Icam4	0,667817	0,403192	0,21096	0,348492
Icam5	0,394278	0,775341	0,186825	0,198401
Il1b	1,39219	0,806906	1,97903	1,03526
Tnf	6,37709	3,32492	4,4016	1,55811
Traf1	1,20497	1,73708	1,04871	0,866203
Traf2	7,87773	9,92028	7,88133	8,17552
Vcam1	11,5451	4,96892	6,38359	4,6591
Mean NFkB target gene expression (C mouse 2)	6,156257917	6,6893408	5,8435163	4,2314522

Mean NKfB target gene expression of control mice (for normalization)	6,48145438	5,4320928	6,129458	5,077115	Data used for normalization
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NFkB target gene	Stat3-ko asymptomatic - mouse 1			
	H1_2w	H1_5w	H1_8w	H1_10w
Bcl2	1,84859	1,37405	2,22196	2,5455
Ddx26b	24,3847	14,5103	18,9545	25,2859
Gadd45b	15,0237	13,2383	17,7276	16,8651
Icam1	6,02257	8,67721	13,1487	9,98603
Icam2	7,9638	4,53143	3,22786	3,20087
Icam4	0,103169	0,332223	0,704791	0,39274
Icam5	0,208837	0,201748	0,653882	0,583823
Il1b	0,580702	0,457103	3,96702	1,136
Tnf	2,92136	1,35334	3,07331	1,51206
Traf1	1,1625	0,770714	2,77694	1,43174
Traf2	5,0872	6,73656	9,53535	8,99125
Vcam1	6,78865	6,65325	6,23863	3,40321
Mean NFkB target gene expression (asymptomatic mouse 1)	6,0079815	4,903019	6,8525453	6,2778519

Normalized mean NFkB target gene expression asymptomatic mouse 1 (to control)	0,926949594	0,9026022	1,1179692	1,2364998	Plotted data
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NFkB target gene	Stat3-ko asymptomatic - mouse 2			
	H2_2w	H2_5w	H2_8w	H2_10w
Bcl2	2,41956	1,23529	2,10121	1,68742
Ddx26b	21,5093	14,3954	24,1115	19,1582
Gadd45b	24,2009	9,00596	17,8458	16,9307

Icam1	11,1511	10,0336	8,47175	10,8174
Icam2	5,5099	3,32845	3,42015	3,09949
Icam4	0,198705	0,211489	0,702098	0,381764
Icam5	0,251389	0,267563	0,24871	0,434685
Il1b	1,24271	0,617243	1,27338	1,35296
Tnf	3,55363	2,06624	2,30199	1,39078
Traf1	2,17314	0,887801	1,51243	0,801295
Traf2	8,19833	6,95227	7,6482	8,33978
Vcam1	5,53173	7,30719	5,17613	4,49481

Mean NFkB target gene expression
(asymptomatic mouse 2)

7,1616995 4,6923747 6,2344457 5,7407737

Normalized mean NFkB target gene
expression asymptomatic mouse 2 (to
control)

1,10495254 0,8638245 1,017128 1,130716 Plotted data

NFkB target gene	Stat3-ko symptomatic - mouse 1			
	D1_2w	D1_5w	D1_8w	D1_10w
Bcl2	2,91098	1,93891	2,06153	1,11461
Ddx26b	23,895	10,6448	23,4599	7,08725
Gadd45b	23,3722	12,3531	23,933	22,7247
Icam1	13,4452	11,8311	8,9785	14,4459
Icam2	5,69212	2,91948	2,05203	5,2818
Icam4	0,404001	0,634164	0,491453	0,418676
Icam5	0,511117	0,504306	0,426347	0,282497
Il1b	0,606394	0,339951	2,10759	256,125
Tnf	3,45202	0,810098	1,49973	3,95179
Traf1	1,84768	1,53122	1,37278	3,14507
Traf2	8,74087	7,9771	7,52432	9,33925
Vcam1	6,08137	4,96439	2,45665	12,669
Mean NFkB target gene expression (symptomatic mouse 1)	7,579912667	4,7040516	6,3636525	28,048795
Normalized mean NFkB target gene expression symptomatic mouse 1 (to control)	1,169477131	0,8659741	1,0382081	5,5245538 Plotted data

NFkB target gene	Stat3-ko symptomatic - mouse 2			
	D2_2w	D2_5w	D2_8w	D2_10w
Bcl2	1,97776	1,43348	1,71809	2,27594
Ddx26b	22,1149	16,3475	15,2385	24,9703
Gadd45b	24,0066	14,7206	36,3763	21,2089
Icam1	12,1641	10,1451	29,7421	8,29952
Icam2	4,82864	2,51071	6,82758	2,72525
Icam4	0,935281	0,570237	0,391841	0,548891
Icam5	0,420714	0,468928	0,272653	0,572899
Il1b	0,974882	0,534944	390,257	9,78346
Tnf	8,10515	1,55804	7,49432	1,09071
Traf1	1,51537	1,3465	9,57717	0,795847
Traf2	8,73212	8,87655	10,819	8,11274
Vcam1	6,86094	4,31381	10,1108	2,56689
Mean NFkB target gene expression (symptomatic mouse 2)	7,71970475	5,2355333	43,235446	6,9126123
Normalized mean NFkB target gene expression symptomatic mouse 2 (to control)	1,19104514	0,9638151	7,053715	1,361524 plotted data

mean normalized nfkb asymptomatic
mean normalized nfkb symptomatic

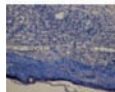
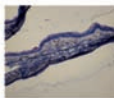
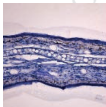
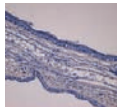
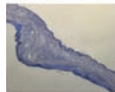
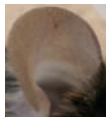
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1,18026114 0,9148946 4,045961 3,443039

Vehicle

LPS

Vehicle

LPS



Asymptomatic Asymptomatic

Asymptomatic

AD flare

