

Critical Dynamics of Peripheral Immune Tolerance: A Treg-Tpex Antagonism Framework for Phase Transition and Immune State Stratification

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Research Article

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Critical Dynamics of Peripheral Immune Tolerance: A Treg-Tpex Antagonism
Framework for Phase Transition and Immune State Stratification
(Version for preprint, revised after critical review)

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Abstract

Paradigm shift: Peripheral immune tolerance is proposed here not as a static steady state but as a critical dynamical system, driven by the continuous accumulation of progenitor-exhausted T cells (Tpex). Under specific conditions, minor perturbations may trigger a nonlinear phase transition toward effector immunity.

Core concept: This paper introduces a conceptual Tolerance Stability Index, $S = F_{\text{Treg}} / N_{\text{Tpex}}$, where F_{Treg} is a composite Treg suppressive function score (integrating CTLA-4 expression, IL-10 secretion, and in vitro suppression IC50), and N_{Tpex} is the antigen-specific Tpex load (cells per immune niche, focusing on CD4+ Tpex with phenotype CD4+CD45RO+PD-1+TCF-1+). As a dimensionless theoretical order parameter, S is proposed to characterize the system's response tendency to chronic antigens with established tolerance. When S approaches a critical threshold S^* , the system enters a highly sensitive state. The following regimes are hypothesized:

$S \gg S^*$ (e.g., before 4-6 years of age) $\rightarrow$ strong tolerance

S slightly above S^* (e.g., young adulthood) $\rightarrow$ requires sufficient danger signals to

trigger a response

$S \leq S^*$ (e.g., self-antigens in the elderly) $\rightarrow$ minor perturbations may shift the system to an effector state

A simplified research estimator $S_{\text{clin}} \approx \text{Treg}\% / \text{Tpex}\%$ is provided strictly for trend monitoring under standardized protocols and is strictly prohibited for clinical decision-making. This framework applies to chronic antigens exposed from infancy with established long-term tolerance (self-antigens, chronic HBV, latent viruses). Responses to novel adult-onset antigens further depend on the naive T-cell repertoire. Detailed formulas and protocols are in the Supplementary Materials.

Mechanistic insight: Specific immune responses are initiated by a hierarchical four-gate model (APC activation, repertoire, suppression, and effector amplification). This framework focuses on Treg-Tpex antagonism within the suppression gate. Persistent chronic antigen stimulation drives T cells to differentiate into Tpex, whose accumulation increases the denominator of S , occupies immune niche space, and consumes homeostatic cytokines (IL-7/IL-15). The elderly may exhibit a "dual-track" pattern: a reduced naive T-cell repertoire limits responses to new antigens, while preserved Tpex reserves support rhythmic fluctuations to chronic/self-antigens (theoretical speculation requiring experimental testing).

Clinical unification: The framework conceptually unifies the age-dependent chronicity of HBV, relapse-remission in autoimmune diseases, and tumor immune escape, thereby offering a theoretical basis for immune interventions in chronic antigen-related diseases.

Conclusion: Tolerance can be viewed as a controllable critical system. S provides a quantitative bridge for immune status monitoring, but clinical translation requires multi-center calibration and prospective validation.

Keywords: Peripheral immune tolerance; Treg-Tpex antagonism; Tolerance Stability Index S; phase transition dynamics; four-gate model; immune niche

Note: A detailed mathematical model of the fast dynamics of the IL-2/Treg axis has been published as a preprint [1] (April 2026, not peer-reviewed). Its core assumptions (e.g., the five-phase IL-2 rhythm) remain experimentally unvalidated. The theoretical year (2026) used in that preprint is part of its modeling context and does not affect the independence of the conclusions drawn in this paper.

I. Core Theoretical Framework: Tolerance as a Critical Dynamical System

I.1 Paradigm Shift: From Static Steady State to Critical System

Classical immunology describes peripheral tolerance as a static steady state maintained by clonal deletion, anergy, and continuous Treg suppression. However, three clinical observations challenge this view:

HBV chronicity rates are ~90% in infants vs. ~5% in adults [2,3];

Autoimmune diseases exhibit relapse-remission cycles [4,5];

Tumor immunoediting follows elimination-equilibrium-escape dynamics [6].

A limitation of classical theories is the absence of quantitative descriptions of system sensitivity and critical thresholds. Here we propose a conceptual framework that models peripheral tolerance as a critical dynamical system: under chronic antigen exposure, Tpex continuously accumulate and antagonize Tregs, driven in part by the IL-2/Treg axis. The fast dynamics of this axis (periodic oscillations over days to weeks) are described in the MICU preprint [1] – although its core assumptions (e.g., the five-phase IL-2 rhythm) remain unvalidated, it provides a reference for fast

dynamics. The present framework focuses on slow variables (months to decades): Tpex accumulation leads to a relative decline in Treg function, eventually driving the Tolerance Stability Index S toward a phase transition.

Path-dependence (hysteresis): After crossing S^* and entering the effector phase, the system does not revert along the original path but forms a new tolerant steady state with a distinct cellular composition. Cellularly, massive Teff activation is followed by activation-induced cell death (AICD) or terminal exhaustion, depleting the Tpex reservoir [7]; meanwhile, Tregs slowly reconstitute [8], restoring S to near S^* and terminating effector function. This new steady state has opposite implications: it represents pathological tolerance (immune escape) in chronic infections/tumors but therapeutic remission in autoimmune diseases.

I.2 The Four-Gate Model for Initiation of Specific Immune Responses

The initiation of a specific effector response is governed by a hierarchical gating cascade:

APC activation gate: Antigen signal strength, danger signal type, and DC subtype collectively determine whether APCs can effectively activate naive T cells.

Repertoire gate: The naive T-cell repertoire size (absolute number and clonal diversity) sets the upper limit of responses to new antigens; the memory T-cell repertoire governs recall response speed; the Tpex reserve determines sustained response capacity to chronic antigens.

Suppression gate: Tregs limit effector initiation via IL-2 competition, the CTLA-4 pathway, and inhibitory cytokines. This gate contains the core variables of S .

Effector amplification condition: The proportion of IL-2-secreting cells (activated

Teffs, memory T cells, Tex-int) in the local microenvironment must reach a critical level, enabling local IL-2 concentration to shift from autocrine to paracrine positive feedback. This threshold is determined by the IL-2 receptor affinity hierarchy (high-affinity $K_d \approx 10$ pM, intermediate-affinity $K_d \approx 10$ nM) [8,9]; a theoretical estimate is ~ 50 pM, but exact values require experimental calibration.

Integration logic: The core of S is the ratio of Treg suppressive function to Tpex load within the suppression gate. APC activation and a sufficient naive repertoire are prerequisites for S to function. The effector amplification condition is a parallel requirement: even if S drops below S^* , insufficient local density of IL-2-secreting cells may still prevent positive feedback.

I.3 Theoretical Metric: Tolerance Stability Index S

Definition (theoretical):

$$S = F_{\text{Treg}} / N_{\text{Tpex}}$$

where

F_{Treg} is a composite Treg suppressive function score (integrating CTLA-4 expression, IL-10 secretion, and in vitro suppression IC50; weights and normalization are theoretical suggestions in the Supplementary Materials).

N_{Tpex} is the antigen-specific Tpex load (cells per immune niche; this framework focuses on CD4⁺ Tpex with phenotype CD4⁺CD45RO⁺PD-1⁺TCF-1⁺).

S is a dimensionless theoretical order parameter, not a directly measurable clinical variable. S^* is the critical threshold, marking the balance between net IL-2 production and consumption in the local microenvironment. Based on idealised MICU

parameters, S^* is theoretically estimated to be $\sim 0.6-1.0$ (derivation in Supplementary Materials), but it is expected to vary across tissue niches (liver, synovium, tumor) and requires empirical calibration.

Simplified research estimator S_{clin} : For exploratory trend monitoring, we define

$$S_{\text{clin}} \approx \text{Treg\%} / \text{Tpex\%}$$

with $\text{Treg\%} = \text{CD4+Foxp3+CD25hi} / \text{total CD4+}$, and $\text{Tpex\%} = \text{CD4+CD45RO+PD-1+TCF-1+} / \text{total CD4+}$.

Critical warnings:

S_{clin} is valid only when Treg suppressive function is positively correlated with Treg% and Tpex load is positively correlated with Tpex% – assumptions that require standardized validation within the same laboratory.

S_{clin} fails if Tregs are functionally defective (e.g., CTLA-4 deficiency, IL-10 loss) or if the measured Tpex are non-antigen-specific; complementary functional assays are then required.

S_{clin} is strictly for within-laboratory trend comparison under identical protocols and is strictly prohibited for individual clinical decision-making.

I.4 Age-Related Tpex Accumulation and Applicability Boundaries

Tpex accumulate under persistent chronic antigen stimulation, affecting S by increasing the denominator, occupying immune niche space, and consuming homeostatic cytokines. Tpex rely on IL-7/IL-15 for survival [10]; their accumulation may deplete local IL-7/IL-15, indirectly weakening Treg survival support [8].

Additionally, Tpex occupation of niche space may competitively inhibit iTreg differentiation [1]. Tpex proportion is generally positively correlated with age [11-13].

"Exposure time – age" synergistic effect (theoretical):

Before 4-6 years: The thymus exports increasing naive T cells and nTregs, marking a "plasticity-shaping period". Chronic antigen exposure during this phase drives naive T cells to differentiate into iTregs, establishing high Treg proportions and robust tolerance. Earlier exposure may lead to more stable tolerance and a later onset of immune clearance.

After 4-6 years: The shaping window narrows, reducing iTreg induction. Additionally, RTE differentiation shifts toward Tpex, accelerating the decline of the Treg/Tpex ratio and making tolerance more vulnerable to disruption.

Dual-track immune responses in the elderly (theoretical speculation requiring testing):

Response to new antigens: Reduced naive T-cell repertoire limits recruitment of antigen-specific T cells, constraining Teff expansion. If the Treg repertoire declines proportionally and the Treg-to-naive T cell ratio remains similar to young adults, a "miniaturized" response is theoretically possible.

Response to chronic/self-antigens: Preserved memory Tpex reserves [11-13] may still support rhythmic fluctuations. Paradoxically, declining Treg function in the elderly might increase autoimmune disease activity.

Core applicable scenarios: Organ-specific chronic antigens persistently exposed from infancy with established long-term immune memory/tolerance (HBV, self-antigens, latent viruses, etc.).

Non-applicable scenarios:

- (1) Novel antigens first encountered in adulthood;
- (2) Systemic autoimmune diseases (e.g., SLE), as core pathology involves the Tfh-B cell axis – outside the Treg-Tpex framework;
- (3) Acute infections;
- (4) Hematological malignancies;
- (5) Immunodeficiency-associated infections.

This framework prioritizes conceptual integration over mechanistic completeness. It is a hypothesis-driven model, not a validated clinical tool.

II. Phase Transition Mechanisms and Unified Interpretation of Clinical Diseases

II.1 Synergistic Effect of Danger Signals and S Value

Danger signals affect the net IL-2 balance by activating APCs, but their effect depends on the current S value of the system:

When $S \gg S^*$ (strong tolerance), even strong danger signals may fail to trigger effector responses against chronic antigens (e.g., HBV immune tolerance phase).

When S is close to or below S^* , danger signals can effectively drive a phase transition.

The relationship between danger signal intensity and S follows a conceptual N-shaped curve (see Supplementary Materials). A recovery branch may exist at intermediate danger levels, corresponding to remission in autoimmune diseases.

Importantly, under danger signals or intermediate-intensity IL-2 stimulation, Tpex can rapidly differentiate into transitional effector cells with low-level IL-2 secretion

(Tex-int) [14,15], which locally recruit and activate Teffs. This is a proposed mechanism by which Tpex load promotes effector responses. Notably, Tpex's "tolerance-weakening" effect (steady-state resource competition, slow variable: months-years) and its "effector-promoting" effect (activation/differentiation, fast variable: days) operate on different timescales – they are complementary, not contradictory, in breaking tolerance.

II.2 Immune State Stratification and Stratified Intervention Strategies

Based on the relationship between S and S^* , we propose a conceptual immune state stratification (see Figure 1 for the S -age trend and intervention windows). This stratification applies only to chronic antigens with established memory/Tpex reserves; for new antigens, naive repertoire size must also be assessed.

Table 1. Theoretical immune state stratification

Immune state	S range	Core prerequisites	Core features	Theoretical intervention rationale
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Pre-threshold steady state	$S > S^*$	Treg suppression dominant	Slow Tpex accumulation; insensitive to perturbations	Reduce S (weaken Treg function / increase Tpex load)
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Dynamic equilibrium	$S \approx S^*$	All four gates functional	Bidirectional plasticity; efficient surveillance	ICB for tumors (enhance effector); block excessive response for autoimmunity
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Effector phase	$S < S^*$	Gating conditions satisfied	Teff expansion; potential AICD	Control inflammation; maximize effector window
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New tolerant steady state	$S \approx S^*$	Effector cells disabled; Treg function restored	Tpex depletion; Treg recovery	Break pathological tolerance (tumors/infections); induce remission (autoimmunity)
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Notes: $S^* \approx 0.6-1.0$ (tissue-specific, requires calibration). The "new tolerant steady state" refers to pathological tolerance (tumors/infections) or therapeutic remission (autoimmunity). The effector phase includes fast IL-2 rhythm sub-phases (temporal division follows the MICU model [1], see Section III.2).

II.3 Conceptual Unification of Clinical Diseases

HBV infection (applicable to chronic HBV carriers exposed from infancy without immunodeficiency):

Immune tolerance phase: $S \gg S^*$, high viral load, low inflammation.

Immune clearance phase: $S < S^*$, viral control coexists with liver damage.

Inactive carrier phase: S slightly above S^* , low viral replication but T_{pex} accumulation.

Active hepatitis phase: $S < S^*$, effector immunity dominant.

Intervention concept: In tolerance phase, lowering S might break tolerance; in clearance phase, balancing effector intensity and inflammatory damage is key.

Autoimmune diseases (applicable to organ-specific diseases like rheumatoid arthritis, type 1 diabetes; not SLE):

Remission: $S > S^*$; active disease: $S < S^*$. Danger-signal-driven S fluctuations may generate relapse-remission cycles [4,16].

Intervention concept: Maintaining $S > S^*$ (e.g., low-dose IL-2 to expand Tregs) as a remission goal; during active disease, controlling inflammation and promoting S

recovery.

Tumor immunoediting:

Elimination phase: $S < S^*$.

Equilibrium phase: $S \approx S^*$ (ICB may be effective).

Escape phase: S returns to near S^* but effector cells are disabled; ICB may have limited efficacy. This framework offers a conceptual explanation for tolerance to tumor-associated antigens (self-antigen nature).

Latent virus infections (EBV, CMV): Persistent low-level antigen stimulation leads to a "low-antigen-pressure, memory-dominant" steady state. Heller et al. (2007) [17] found functionally intact EBNA1-specific CD4⁺ T cells in healthy EBV carriers; Sturmlechner et al. (2025) [18] showed no exhaustion in elderly EBV-specific CD8⁺ T cells; Yamada et al. (2023) [19] identified antigen load as a key factor. Thus, EBV/CMV latency is proposed as a pre-threshold steady state with S slightly above S^* .

III. Testable Predictions and Research Translation Directions

III.1 Core Testable Predictions

Subclinical T-cell oscillations in HBV tolerance phase: High-throughput TCR sequencing with time-series analysis (biweekly for 3 months) defining 7-30-day cycles as oscillations. Partial correlation analysis adjusting for age, viral load, and liver injury should assess the relationship between oscillation amplitude and S_{clin} . Expected result: amplitude negatively correlated with S_{clin} .

Tpex frequency as a disease warning marker: A prospective cohort ($n \geq 200$, 5 years) enrolling chronic HBV patients in the tolerance phase, monitoring peripheral blood Tpex% every 6 months. The age-matched healthy control 95% CI upper limit serves as the threshold. Cox regression adjusting for confounders. Expected result: Tpex% above threshold yields $HR > 3.0$.

Negative correlation between thymic output and Tpex accumulation: Correlation analysis using sjTREC and Tpex frequency ($n \geq 100$). Expected result: significant negative correlation ($r < -0.4$, $p < 0.01$).

S_clin-guided stratified intervention: Prospective studies in autoimmune/tumor patients stratifying by S_clin median. Expected result: lower autoimmune relapse rates with S_clin above median; better ICB efficacy in tumors with S_clin near S^* .

sCD25 as a warning marker: Continuous monitoring of serum sCD25 in autoimmune high-risk populations, with twice the baseline median as the threshold. Expected result: persistent elevation predicts higher relapse risk within 3-6 months (log-rank test, $p < 0.05$).

III.2 Research Translation Tools

STRICT WARNING: These tools are for research exploration only and are absolutely prohibited for routine clinical practice. All intervention strategies below are theoretical, unvalidated, and for reference only.

Research monitoring: Use S_clin for macro-stage monitoring and measure IL-2, sCD25, etc., to identify micro-rhythm sub-phases. Micro-rhythm temporal division (activation/effector, contraction, suppression/trough, re-equilibrium) follows the MICU model [1]; kinetic parameters require experimental determination.

Macro-micro combined stratified intervention strategies (theoretical, unvalidated):

Table A. Infection / Tumor (goal: break tolerance, enhance/prolong effector response)

Micro-rhythm sub-phase	IL-2 level	Teff/Treg status	Macro S state	Applicable intervention (theoretical)	Contraindicated intervention
Activation/effector	≥ 50 pM	Teff positive feedback	$S < S^*$ (effector phase)	IFN- α , ICB, IL-2	Treg agonists
Contraction	10-50 pM (declining)	Teff apoptosis, Treg activation	$S < S^*$ or rising	Anti-CTLA-4, anti-CD25	Any IL-2, IFN- α
Suppression/trough	1-10 pM	Treg dominant	$S > S^*$ or near S^*	ICB, combination vaccines	Low-dose IL-2
Re-equilibrium	< 1 pM	Immune silent	$S > S^*$	Monitoring	None

Note: IL-2 is contraindicated in the contraction phase because Treg activation here depends on IL-2; exogenous IL-2 would promote Treg proliferation and weaken subsequent effector responses.

Table B. Autoimmune diseases (goal: inhibit Teff, promote contraction, induce Treg-mediated remission)

Micro-rhythm sub-phase	IL-2 level	Teff/Treg status	Macro S state	Applicable intervention	Contraindicated intervention
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				n (theoretical)	
Activation/effecto r	≥ 50 pM	Teff positive feedback	$S < S^*$ (active phase)	Anti-IL-2, anti-CD25, calcineurin inhibitors	IL-2, ICB
Contraction	10-50 pM (declining)	Teff apoptosis , Treg activation	$S < S^*$ or rising	Low-dose IL-2	Anti-Treg therapy, ICB
Suppression/troug h	1-10 pM	Treg dominant	$S > S^*$ (remissio n phase)	Low-dose IL-2	Anti-Treg therapy, ICB
Re-equilibrium	< 1 pM	Immune silent	$S > S^*$	Monitoring	None

Feasibility note: Low-dose IL-2 expands Tregs in autoimmune trials (e.g., SLE, type 1 diabetes). Anti-CTLA-4 (ipilimumab) and anti-PD-1 are approved for cancer. However, the macro-micro stratified strategy lacks prospective validation – all are theoretical.

Future direction: Multi-center prospective cohorts (≥ 30 patients per center, total ≥ 200) collecting S_{clin} and IL-2 rhythm data, using disease flare/efficacy as endpoints to build a macro-micro stratification model. S^* should be calibrated by tissue type (liver, tumor, synovium, etc.).

IV. Conclusions and Boundaries

IV.1 Core Contributions

Proposes the Tolerance Stability Index S as a conceptual quantitative framework for immune tolerance to infant-exposed chronic antigens.

Constructs a "four-gate model" of immune response initiation, clarifying S 's central role in the suppression gate.

Unifies age-related immune changes and three clinical paradoxes within a Treg-Tpex critical dynamics framework.

Precisely delineates the framework's core applicability and boundaries to prevent overgeneralization.

IV.2 Paradigm Significance and Translation Pathways

Tolerance is conceptualized not as a "solid fortress" but as a "critical dynamic surface". An S -based monitoring concept could enable predictive immune modulation. The translation path requires multi-center calibration of S^* and prospective validation of intervention strategies.

IV.3 Theoretical Boundaries and Model Limitations

Applicable scenarios: Organ-specific chronic antigens persistently exposed from infancy with established long-term immune memory/tolerance (HBV, self-antigens, latent viruses, etc.).

Non-applicable scenarios:

- (1) Novel adult-onset antigens;
- (2) Systemic autoimmune diseases (SLE);

- (3) Acute infections;
- (4) Hematological malignancies;
- (5) Immunodeficiency-associated infections.

Model limitations:

S_{clin} weights are uncalibrated; the Treg-function-Treg% proportionality assumption fails when Tregs are functionally defective or Tpex are terminally exhausted.

Indirect regulators (B cells, macrophages, MDSCs) are under-incorporated.

Peripheral blood-local niche consistency requires clarification.

The naive T-cell repertoire (an independent dimension) is excluded from the main framework for simplicity.

Microbiome, sex differences, and tissue niche heterogeneity are not addressed (future research priorities).

Figure Legends

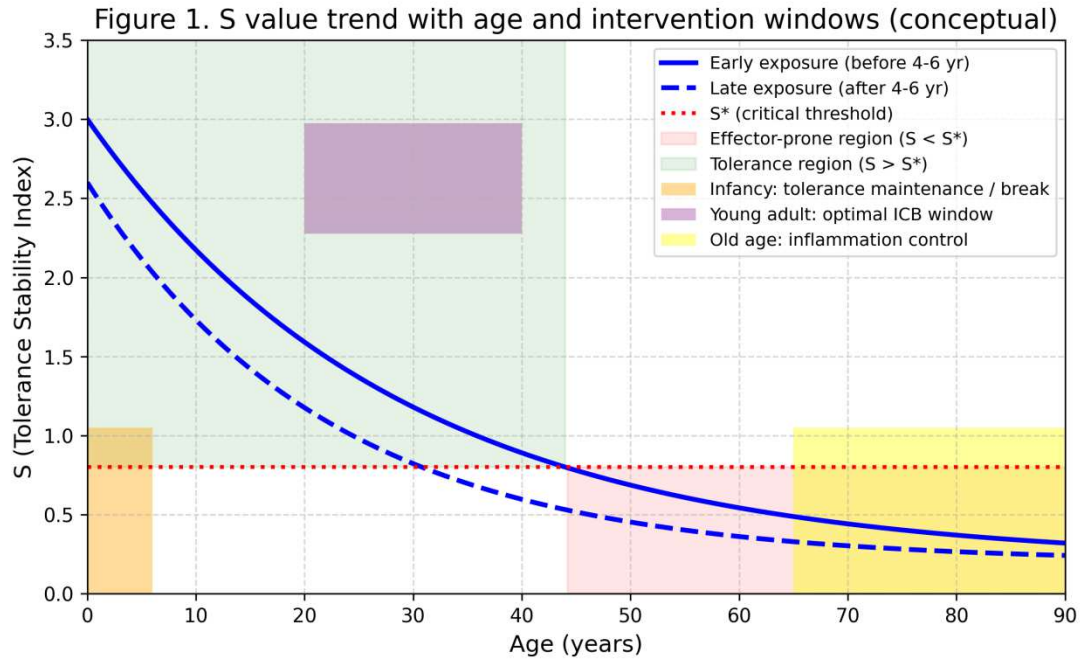


Figure 1. Trend of S with age and intervention windows

Fitted conceptually from literature [11-13]. The curve shows: infancy ($S \approx 2-3$, $S \gg S^*$, pre-threshold steady state), young adulthood ($S \approx 0.5-1$, $S \approx S^*$, dynamic equilibrium), and old age ($S \approx 0.2-0.5$, $S < S^*$, effector-prone). The curve reflects an "exposure time-age" synergy: early exposure (solid line, antigen exposure before 4-6 years) $\rightarrow$ high S, long tolerance; late exposure (dashed line, exposure after 4-6 years) $\rightarrow$ faster S decline. Intervention windows are marked: infancy tolerance maintenance (lower S to break tolerance), young adulthood optimal ICB window (tumor equilibrium phase), and old age inflammation control (block excessive response).

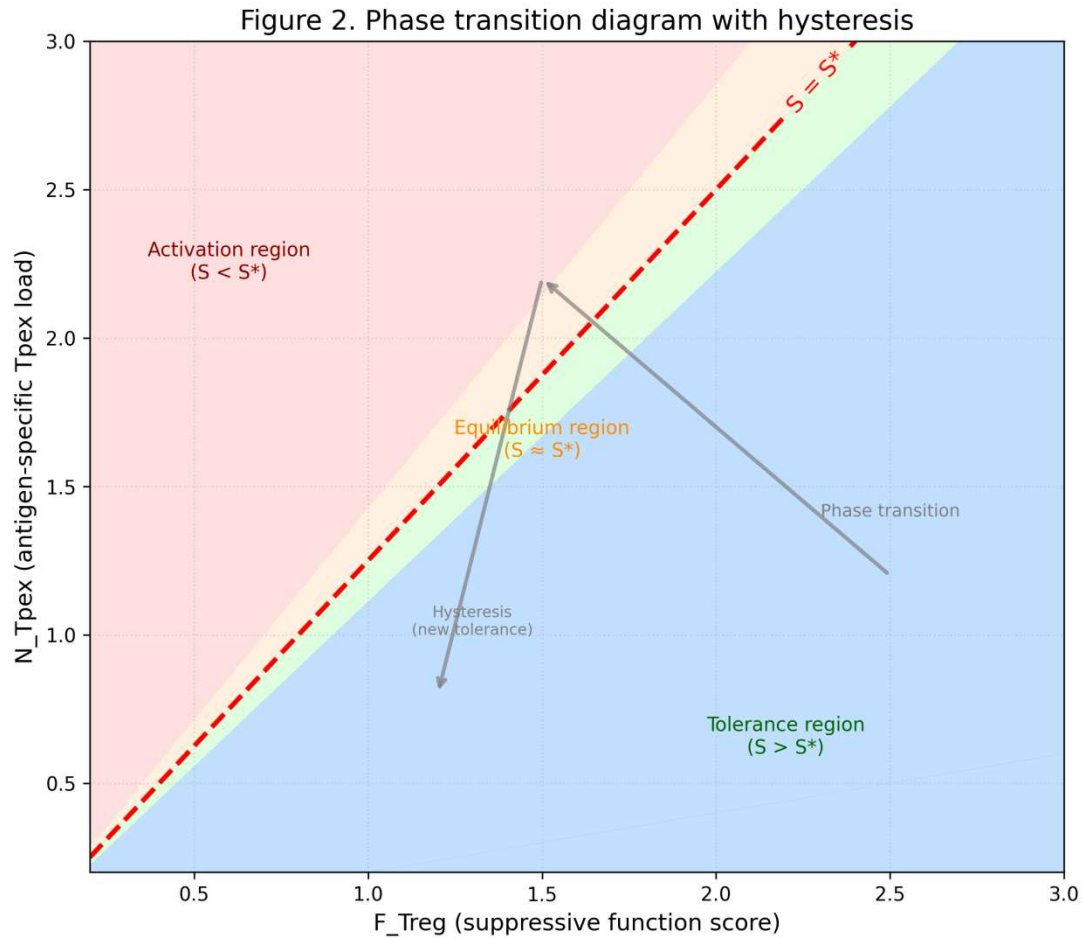


Figure 2. Phase transition diagram

Horizontal axis = F_{Treg} (suppressive function score); vertical axis = N_{Tpex} (antigen-specific Tpex proportion). The dashed line $S = S^*$ is the critical threshold. Tolerance region ($S > S^*$): Treg suppression dominant, Tpex accumulation. Equilibrium region ($S \approx S^*$): bidirectional plasticity, efficient surveillance. Activation region ($S < S^*$): Teff expansion dominant. The gray dashed arrow indicates hysteresis: from the activation region to a new tolerance region with Treg-dominant, Tpex-depleted cellular composition. The original (Tpex-rich, strong Treg function) and new (Tpex-depleted, Treg-recovered) tolerance states differ in cellular composition, reflecting phase transition irreversibility.

References (ordered by first citation in text)

[1] Zhang Y. Intrinsic Molecular Logic Underpins Adaptive Immune Rhythms: An

IL-2/Treg Axis-Centered MICU Model [Preprint]. Research Square; 2026. DOI: 10.21203/rs.3.rs-9549819/v1.

[2] Kennedy PTF, et al. Advances in Hepatitis B Virus Infection and Immune Tolerance. *Gastroenterology*. 2012;143(4):966-975. DOI: 10.1053/j.gastro.2012.07.004.

[3] Lok ASF, McMahon BJ. Chronic Hepatitis B: Update 2009. *Hepatology*. 2009;50(3):661-662. DOI: 10.1002/hep.23098.

[4] Lublin FD, et al. Defining the Clinical Course of Multiple Sclerosis. *Neurology*. 2014;83(3):278-286. DOI: 10.1212/WNL.0000000000000560.

[5] Elkoshi Z. Relapse-Remission Dynamics in Autoimmune Diseases. *Front Immunol*. 2025;16:1522356. DOI: 10.3389/fimmu.2025.1522356.

[6] Schreiber RD, et al. Cancer Immunoediting: Integrating Immunity's Roles in Cancer Suppression and Promotion. *Science*. 2011;331(6024):1565-1570. DOI: 10.1126/science.1203486.

[7] Andreato F, et al. The Role of Exhausted T Cells in Immune Tolerance. *Sci Transl Med*. 2024;16(729):eadi1572. DOI: 10.1126/scitranslmed.adi1572.

[8] Mannie MD, et al. Treg-Mediated Suppression in Immune Homeostasis. *Front Immunol*. 2023;14:1123445. DOI: 10.3389/fimmu.2023.1123445.

[9] Malek TR. The Biology of Interleukin-2. *Annu Rev Immunol*. 2008;26:453-479. DOI: 10.1146/annurev.immunol.26.021607.090242.

[10] Vang KB, et al. Homeostatic Cytokines in T Cell Survival. *J Immunol*.

2021;206(8):1789-1799. DOI: 10.4049/jimmunol.2001039.

[11] Kumar BV, et al. Age-Related Changes in Human T Cell Repertoire. *Immunity*. 2018;48(2):202-213. DOI: 10.1016/j.immuni.2018.01.014.

[12] Pawelec G. Immunosenescence and T Cell Function. *Semin Immunol*. 2018;40:10-18. DOI: 10.1016/j.smim.2018.09.003.

[13] Mittelbrunn M, Kroemer G. Immune Aging and Metabolic Regulation. *Nat Rev Immunol*. 2021;21(5):315-329. DOI: 10.1038/s41577-020-00490-8.

[14] Wherry EJ, Kurachi M. Molecular and Cellular Insights into T Cell Exhaustion. *Nat Rev Immunol*. 2015;15(8):486-499. DOI: 10.1038/nri3867.

[15] Huang AC, et al. The Role of T Cell Exhaustion in Cancer Immunity. *Nature*. 2017;545(7652):60-65. DOI: 10.1038/nature22072.

[16] Steinman L. Mechanisms of Autoimmune Disease. *Annu Rev Immunol*. 2014;32:257-281. DOI: 10.1146/annurev-immunol-032713-120227.

[17] Heller KN, et al. EBV-Specific T Cell Function in Healthy Carriers. *Blood*. 2007;109(3):1138-1146. DOI: 10.1182/blood-2006-07-034876.

[18] Sturmlechner I, et al. EBV-Specific T Cells in Elderly Carriers. *Nat Commun*. 2025;16(1):6394. DOI: 10.1038/s41467-025-61934-w.

[19] Yamada M, et al. Antigen Load and T Cell Response in Latent Viral Infections. *Am J Transplant*. 2023;23(8):1145-1158. DOI: 10.1016/j.ajt.2023.04.022.

Supplementary Materials

(The Supplementary Materials are provided as a separate document. They contain theoretical quantification formulas, danger load framework, phase transition kinetic derivations, Python code, ethical guidelines, and high-resolution versions of Figures 1-2.)

Declarations

Conflict of Interest: The author declares no competing interests.

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Ethics Approval: This is a theoretical framework study; no human or animal experiments were involved, so ethics approval is not applicable.

Data Availability: No new primary data were generated. All analyses are based on previously published data and the author's mathematical model, fully described in the text and Supplementary Materials. The code is available from the corresponding author upon reasonable request.

Acknowledgements: AI-assisted tools (ChatGPT, iFlytek) were used solely for language polishing, formatting adjustment, and literature retrieval during manuscript preparation. These tools did not participate in theoretical framework construction, formula derivation, intervention strategy design, data analysis, or scientific conclusion formation. All scientific content and final conclusions are the sole responsibility of the author.

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End of Revised Manuscript

Supplementary Materials

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Statement

All experimental protocols, validation methods, and numerical simulations described in this Supplementary Material are theoretical suggestions. The author's institution lacks the necessary experimental platforms and ethical approval qualifications. These protocols are provided solely for reference by qualified independent research institutions. The author has not conducted any experiments involving animals or human specimens, and this material contains no primary experimental data.

Risk warning: The experimental protocols herein have not been validated in animals or humans; uncertainties regarding technical feasibility and ethical compliance remain. They are strictly prohibited for direct clinical application or commercial use.

Collaboration note: Artificial intelligence tools (ChatGPT, iFlytek) were used only for language polishing, format standardization, and literature retrieval assistance. The core theoretical construction, formula derivation, experimental protocol design, and academic judgments were completed independently by the author.

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Theoretical Quantification System for S Value: Formula and Weight Calibration

Conceptual Framework for Danger Load (D): Quantification and Dynamics

Numerical Simulation of Tolerance Phase Transition Dynamics (with Python and MATLAB code)

Validation Protocol for Critical Tpex Proportion (Theoretical Suggestions)

Supplementary Figures (for materials not appearing in the main text)

Ethical Compliance and Standardization Guidelines

References

Theoretical Quantification System for S Value: Formula and Weight Calibration

1.1 Definition of Theoretical S (consistent with Section I.3 of the main text)

The Tolerance Stability Index S is defined as:

$$S = F_Treg / N_Tpex$$

where

F_Treg: comprehensive Treg suppressive function score (dimensionless, theoretical construct)

N_Tpex: antigen-specific Tpex load (cells per immune niche)

1.2 Quantification Formula for F_Treg (theoretical proposal)

$$F_Treg = w1 * (CTLA-4 \text{ MFI ratio}) + w2 * (IL-10 \text{ secretion ratio}) + w3 * (IC50 \text{ ratio}) \\ + w4 * (Treg\% \text{ ratio})$$

Each component is normalized to the median of healthy controls.

Initial weight proposals (theoretical, requiring multi-center calibration):

$$w1 = 0.3, w2 = 0.2, w3 = 0.3, w4 = 0.2.$$

1.3 Tissue-Peripheral Blood Conversion for N_Tpex

$$N_Tpex(\text{tissue}) = a * (Tpex\% \text{ in blood}) + b$$

Coefficients a and b are to be obtained by linear regression using paired samples ($n \geq 30$), with a target $R^2 \geq 0.7$.

Theoretical expectation: a typically in the range 1.5-4.0, b in +/-1.0, but strong dependence on tissue and protocol is expected.

1.4 Failure Criteria for S_clin

The simplified research estimator $S_clin \approx Treg\% / Tpex\%$ is invalid under any of the following conditions:

Treg functional exhaustion: CTLA-4 MFI ratio < 0.5 and IC50 ratio < 0.3

Tpex terminal exhaustion: TCF-1 negative and PD-1 MFI > 2 -fold the median of healthy controls

Note: These thresholds are illustrative and require experimental validation.

Conceptual Framework for Danger Load (D): Quantification and Dynamics

2.1 Components of D

$$D = k_1 * \text{PAMP_score} + k_2 * \text{DAMP_score} + k_3 * \text{Inflammatory cytokine score}$$

Initial theoretical weights: $k_1 = 0.4$, $k_2 = 0.4$, $k_3 = 0.2$.

2.2 Conceptual Grading Thresholds (theoretical)

Grade D range Hypothesized biological effect

D1 0.0-0.3 APC activation

D2 0.3-0.7 Naive T-cell activation, small amount of IL-2

D2' 0.7-1.0 Initiation of Tpex differentiation

D3 1.0-1.5 Effector positive feedback ($\text{IL-2} \geq 50 \text{ pM}$)

D4 >1.5 Runaway inflammation

The conceptual N-shaped relationship between danger signal intensity and S value is shown in Supplementary Figure 1 (see Section 5).

2.3 Kinetic Equation (theoretical simplification)

$$dS/dt = -\alpha * D * (S - S^*) + \beta * (S - S^*) * (D - D2')_+$$

where S^* is the critical threshold (see main text), $D2' = 0.7$, and $(x)_+ = \max(0, x)$.

This equation is a conceptual representation; the numerical simulations in Section 3 use a more detailed two-variable model.

Numerical Simulation of Tolerance Phase Transition Dynamics (with Python and MATLAB code)

3.1 Core Equations and Parameter Definitions (theoretical model)

The following system models the slow dynamics of S and the fraction z of differentiated T_{pex} :

$$dS/dt = r * S * (1 - S/K) - \text{gamma} * D * (S - S^*) + \text{lambda} * z * (S - S^*)$$

$$dz/dt = \text{eta} * (D - D2')_+ * (1 - z) - \text{delta} * z$$

Parameter definitions and theoretical choices (time unit: day; all rates per day):

Parameter	Meaning	Theoretical value	(exploratory)	Justification
-----------	---------	-------------------	---------------	---------------

r	Intrinsic growth rate of S	0.05	Assumed slow T_{reg} proliferation in chronic infection
-----	------------------------------	------	---

K	Carrying capacity of S	3.0	Theoretical upper bound
-----	--------------------------	-----	-------------------------

gamma	Danger-induced tolerance breaking coefficient	0.3	Chosen to obtain quasi-steady state within ~100 days in simulation (exploratory)
----------------	---	-----	--

S^*	Critical threshold	0.8	Derived from MICU assumptions (see main text)
-------	--------------------	-----	---

lambda	T_{pex} regulation coefficient	0.2	Antagonism strength
-----------------	----------------------------------	-----	---------------------

eta	T_{pex} differentiation rate	0.15	Efficiency of danger-induced differentiation
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delta	T_{pex} decay rate	0.03	Natural clearance
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$D2'$	Threshold for differentiation initiation	0.7	From Section 2.2
-------	--	-----	------------------

3.2 Python Simulation Code

Save the following code as `MICU_S_simulation.py` and run in Python 3.7+ (requires `numpy`, `scipy`, `matplotlib`). The figure will be saved automatically.

```

python
# MICU_S_simulation.py

# Numerical simulation of tolerance phase transition dynamics: S value dynamics
under different danger signals

# Time unit: day; all rate parameters are per day


import numpy as np
from scipy.integrate import solve_ivp
import matplotlib.pyplot as plt


# Parameter settings (chronic infection scenario)
params = {
    'r': 0.05, 'K': 3.0, 'gamma': 0.3, 'S_star': 0.8,
    'lam': 0.2, 'eta': 0.15, 'delta': 0.03, 'D_thr': 0.7
}


def micu_ode(t, y, params, D):
    S, z = y
    D_plus = max(D - params['D_thr'], 0.0)
    dSdt = (params['r'] * S * (1 - S/params['K'])
            - params['gamma'] * D * (S - params['S_star'])
            + params['lam'] * z * (params['S_star'] - S))
    dzdt = params['eta'] * D_plus * (1 - z) - params['delta'] * z
    return [dSdt, dzdt]


def simulate(params, D, t_max=200, init_S=2.5, init_z=0.05):
    sol = solve_ivp(lambda t, y: micu_ode(t, y, params, D),
                    (0, t_max), [init_S, init_z],
                    t_eval=np.linspace(0, t_max, 1000),
                    method='RK45', rtol=1e-6)

```

```

    return sol.t, sol.y[0], sol.y[1]

if __name__ == "__main__":
    D_vals = [0.2, 0.6, 1.0, 1.5]
    colors = ['blue', 'green', 'orange', 'red']
    plt.figure(figsize=(10,4))
    for Dv, col in zip(D_vals, colors):
        t, S, _ = simulate(params, Dv)
        plt.plot(t, S, color=col, label=f'D={Dv}')
    plt.axhline(params['S_star'], color='k', linestyle=':', label='S*')
    plt.xlabel('Time (days)')
    plt.ylabel('S value')
    plt.legend()
    plt.title('S dynamics under different danger signals (theoretical simulation)')
    plt.tight_layout()
    plt.savefig('S_dynamics.png', dpi=300)
    # plt.show()    # commented out for headless environments

```

3.3 MATLAB Simulation Code

Save the following code as MICU_S_simulation.m and run in MATLAB (R2015b or later). The figure will be saved automatically.

```

matlab
% MICU_S_simulation.m
% Numerical simulation of tolerance phase transition dynamics
% Time unit: day; all rate parameters are per day

clear; clc; close all;

% Parameter settings

```

```

params.r = 0.05; params.K = 3.0; params.gamma = 0.3; params.S_star = 0.8;
params.lam = 0.2; params.eta = 0.15; params.delta = 0.03; params.D_thr = 0.7;

% Differential equation
odefun = @(t, y, D) [
    params.r * y(1) * (1 - y(1)/params.K) ...
        - params.gamma * D * (y(1) - params.S_star) ...
        + params.lam * y(2) * (params.S_star - y(1));
    params.eta * max(D - params.D_thr, 0) * (1 - y(2)) - params.delta * y(2)
];

% Simulation function
simulate = @(D, tspan) ode45(@(t, y) odefun(t, y, D), tspan, [2.5; 0.05]);

% Different danger signal levels
D_vals = [0.2, 0.6, 1.0, 1.5];
tspan = [0 200];
colors = {'b','g','m','r'};
figure; hold on;
for i = 1:length(D_vals)
    [t, y] = simulate(D_vals(i), tspan);
    plot(t, y(1,:), 'Color', colors{i}, 'DisplayName', sprintf('D=%.1f', D_vals(i)));
end
yline(params.S_star, 'k--', 'S*');
xlabel('Time (days)'); ylabel('S value');
legend show; title('S dynamics under different danger signals (theoretical simulation)');
saveas(gcf, 'S_dynamics_matlab.png');

```

The simulation results are presented in Supplementary Figure 2 (see Section 5).

Validation Protocol for Critical Tpex Proportion (Theoretical Suggestions)

4.1 Animal Model

Model: C57BL/6 mice chronically infected with LCMV Clone 13

Groups: control (saline), low dose (10^2 Tpex), medium dose (10^3 Tpex), high dose (10^4 Tpex); $n \geq 6$ per group

Procedure: Tail vein adoptive transfer of sorted antigen-specific Tpex (CD8+PD-1+TCF-1+, purity $\geq 90\%$)

Readouts: Flow cytometry for IFN- γ + CD8+ T cell proportion in spleen/liver at 7-14 days; qPCR for viral load

Statistics: One-way ANOVA; $p < 0.05$ considered significant

Theoretical expectation: Medium- and high-dose groups show significantly higher effector T-cell proportions than the low-dose group ($p < 0.01$), suggesting a hypothetical critical tissue density of approximately 10^3 cells per mm^2 of liver section (by multiplex immunofluorescence).

4.2 Human Samples (ethical requirements)

Samples: Liver biopsy tissue from chronic HBV patients ($n \geq 30$), age-matched healthy controls ($n \geq 30$)

Assay: Multiplex immunofluorescence to quantify Tpex density in the portal area (cells per mm^2)

Analysis: Use the 95% confidence interval upper limit of Tpex density in healthy controls as a threshold; logistic regression to calculate risk of ALT elevation (adjusting for age, sex, viral load)

Theoretical expectation: Tpex density above the threshold yields an odds ratio for ALT elevation > 3 (95% CI not including 1), supporting Tpex as a potential warning marker.

4.3 Literature Support

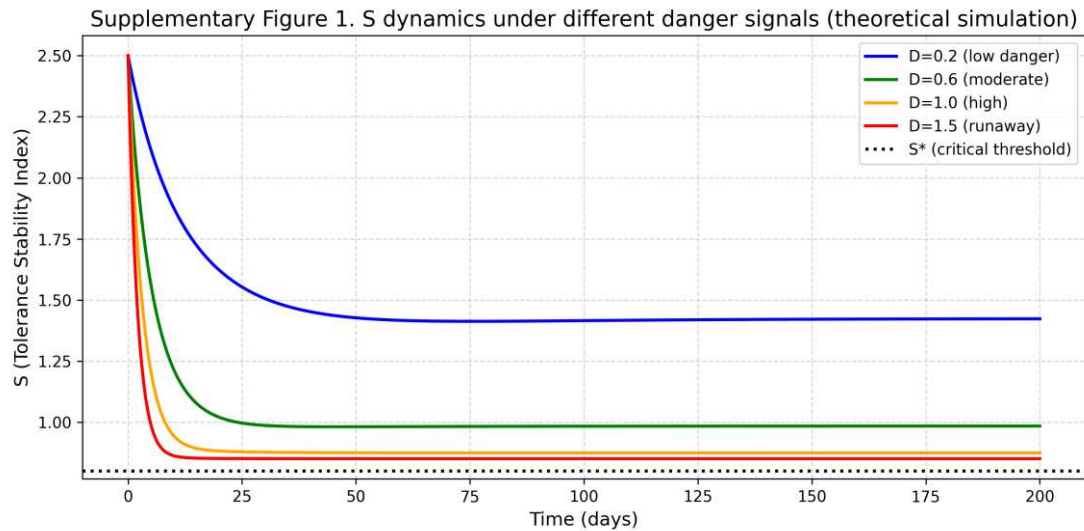
[S1] Beltra JC, et al. Developmental relationships of four exhausted CD8⁺ T cell subsets reveals underlying transcriptional and epigenetic landscape control mechanisms. *Immunity*. 2020;52(5):825-841.

[S2] Utzschneider DT, et al. T cell factor 1-expressing memory-like CD8⁺ T cells sustain the immune response to chronic viral infection. *Nat Immunol*. 2016;17(8):966-976.

Supplementary Figures

The following supplementary figures are not duplicated from the main text. High-resolution versions of main text Figures 1 and 2 are available from the corresponding author upon request.

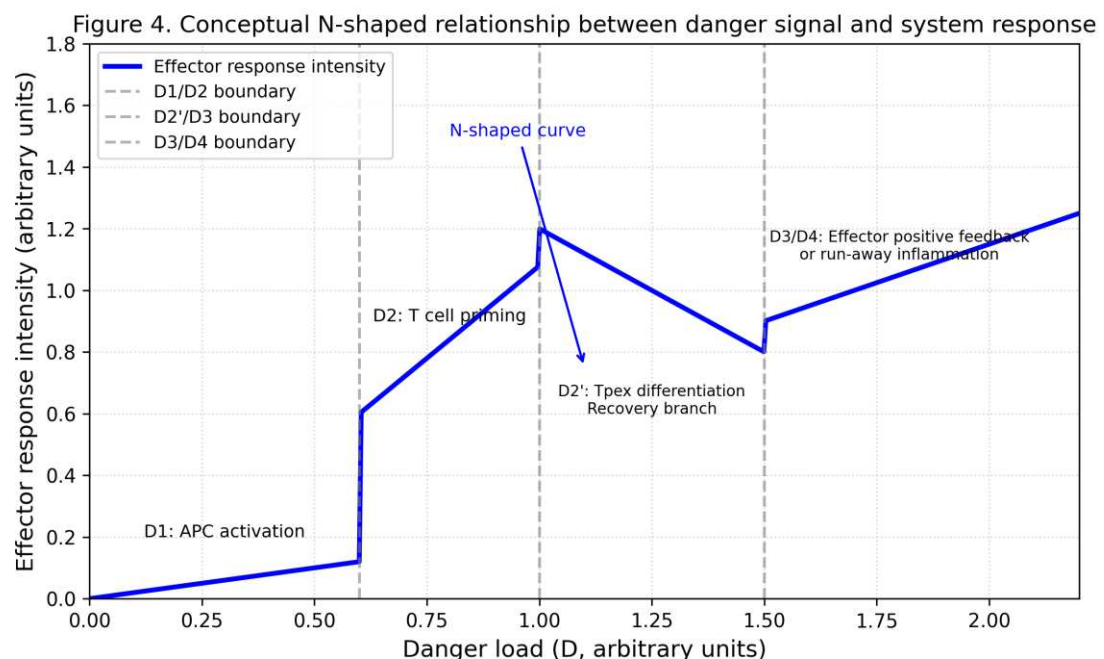
Supplementary Figure 1. Conceptual N-shaped relationship of danger signal vs. S
(Insert image: Supplementary_Figure1_N_curve.png)



Caption: The curve illustrates the hypothesized relationship between danger signal load D (arbitrary units) and the S value. Graded thresholds D_1 , D_2 , D_2' , D_3 , D_4 are marked. A recovery branch (shaded area between D_2 and D_3) corresponds to a transient S rebound, representing the biological window for autoimmune disease remission. The horizontal dashed line indicates S^* . This figure is referenced in Section 2.2 of the main text and Section 2.2 of the Supplementary Materials.

Supplementary Figure 2. Simulated S dynamics under different danger signal loads

(Insert image: Supplementary_Figure2_S_dynamics.png)



Caption: Numerical simulation of S value over time (days) under four different danger signal levels ($D = 0.2, 0.6, 1.0, 1.5$) using the two-variable model described in Section 3.1. The black dashed line indicates the critical threshold $S^* = 0.8$. Higher danger signals ($D \geq 1.0$) drive S below S^* , triggering a phase transition. This figure is generated by the Python/MATLAB code in Sections 3.2 and 3.3.

Note: Main text Figures 1 and 2 (S-age trend and phase transition diagram) are embedded in the main text and are not reproduced here.

Ethical Compliance and Standardization Guidelines

If the experimental protocols described in this material are to be implemented in the future, the following must be ensured:

Animal experiments: Comply with local institutional guidelines for laboratory animal welfare and obtain approval from the Institutional Animal Care and Use Committee (IACUC).

Human studies: Comply with the Declaration of Helsinki and the applicable national regulations (e.g., in China, the "Measures for Ethical Review of Biomedical Research Involving Humans"), and obtain ethics committee approval and written informed consent from all participants.

Data sharing: Adhere to the CC BY 4.0 license, cite the source, and do not use for commercial purposes.

References

From main text:

[8] Mannie MD, et al. Treg-Mediated Suppression in Immune Homeostasis. Front

Immunol. 2023;14:1123445. DOI: 10.3389/fimmu.2023.1123445.

Supplementary-specific:

[S1] Beltra JC, et al. Developmental relationships of four exhausted CD8⁺ T cell subsets reveals underlying transcriptional and epigenetic landscape control mechanisms. *Immunity*. 2020;52(5):825-841. DOI: 10.1016/j.immuni.2020.04.008.

[S2] Utzschneider DT, et al. T cell factor 1-expressing memory-like CD8⁺ T cells sustain the immune response to chronic viral infection. *Nat Immunol*. 2016;17(8):966-976. DOI: 10.1038/ni.3469.

End of Supplementary Materials