

# Transient selection in multi-cellular immune networks

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We analyze the dynamics of a multi-clonotype naive T-cell population competing for survival signals from antigen-presenting cells. We find that this competition provides with an efficacious selection of clonotypes, making the less able and more repetitive get extinct. We uncover the scaling principles for large systems the extinction rate obeys and calibrate the model parameters to their experimental counterparts. For the first time we estimate the physiological values of the T-cell receptor – antigen presentation profile recognition probability and T-cell clonotypes niche overlap. We demonstrate that, while the ultimate state is a stable fixed point, sequential transients dominate the dynamics over large timescales that may span over years, if not decades, in real time. We argue that what is currently viewed as 'homeostasis' is a complex sequential transient process, while being quasi-stationary in the total number of T-cells only. The discovered type of sequential transient dynamics in large random networks is a novel alternative to the stable heteroclinic channel mechanism.

The main functional role of adaptive immune system is to mount response to novel pathogens and form immune memory to target the cognate ones more efficiently. Its regulation is one of the central problems of immunology [1, 2]. Experimental data show that the number of naive T-cells (a type of pre-memory state lymphocytes) remains much the same in adult healthy humans, that is about  $10^{11} - 10^{12}$  of T-cells divided into  $10^7 - 10^8$  clonotypes (subsets with identical recognition properties) [3]. Theoretical arguments and experimental evidence indicate that the mechanism of regulation in the absence of infection could be the competition between T-cells for 'survival stimuli' from specialized antigen-presenting cells (APC) (dendritic cells, typically) [4, 5].

The huge number of T-cells is not sufficient for a reliable functioning of the immune system: it requires the diversity of recognition patterns of T-cell clonotypes (TCCs) to combat pathogens in all their potential variety. Naive T-cells develop in thymus and migrate to the periphery, their antigen recognition profiles remaining random-like [6]. Simple combinatorial arguments show that a massive excess in T-cell numbers is needed to cover the pathogen space unless further selection takes place in peripheral organs [7, 8].

The physical picture of the clonotype selection process is the following. Naive T-cells from each clonotype recognize its own set of antigen-presenting profiles (APPs) (see Fig.1). Moving in the lymph node T-cells interact with APCs (much at random) [9] and, should recognition occur, get a stimulus for division. Cells also have their characteristic lifetime and die occasionally. The clonotype gets extinct if its proliferation is not rapid enough to replenish losses, be it due to its low APP

T-cell clonotypes    Antigen presentation profiles

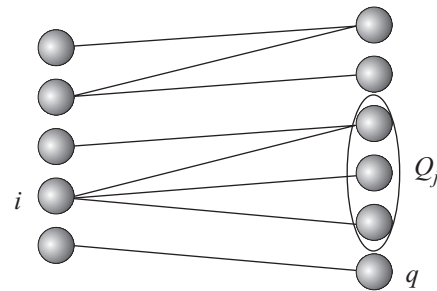


Fig.1. Schematic representation of T-cell – antigen presentation profile recognition network as a random bipartite graph

recognition ability or strong competition with the other clonotypes for access to APCs.

A powerful mathematical approach is modeling TCC competition as a Markovian birth and death process, where the TCC-APP-specific birth rates are downscaled by the number of competing cells and cell-specific death rates are constant [7]. Such model, however, poses serious challenges for analytical and computational studies. The progress has been possible only to the extent of showing (in the mean-field approximation) that the more competing clonotypes get extinct faster [7, 10], existence of the limiting conditional probability distribution and stochastic extinction of all clonotypes on the exponentially long timescale [7, 11]. The most intriguing questions of the origin and stability of a stationary ('homeostatic') T-cell pool size, distributions of the clonotype sizes and numbers in ensembles of realistic size, the dynamics and timescales of selection process remain to be answered.

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To make an advance here we develop and study a deterministic dynamical model of multi-clonotype T-cell population competing for survival signals from antigen-presenting cells. We find that this competition induces a massive extinction of clonotypes with narrower and more overlapping recognition profiles. We uncover the scaling of extinction rate to large systems and calibrate the model parameters to their experimental counterparts. For the first time we estimate the physiological values of the T-cell receptor – APP recognition probability and TCC niche overlap. We demonstrate that, while the ultimate state is a stable fixed point, sequential transients dominate the dynamics over large timescales that may span over years, if not decades, in real time. We argue that the homeostasis is a complex sequential transient process, while being quasi-stationary in the total number of T-cells only.

The theory of multivariate stochastic processes yields the dynamical equations for mean sizes of clonotypes with kinetic coefficients corresponding to those of the birth and death processes [12]:

$$\dot{n}_i = n_i \left( -\mu + \sum_{q \in Q_i} \frac{\gamma}{n_i + \sum_{j \neq i: q \in Q_j} n_j} \right), \quad i = \overline{1, N}. \quad (1)$$

Here  $n_i$  is the size of the  $i$ -th clonotype population,  $\mu$  is the aggregate death/migration to the memory T-cells pool/influx from thymus rate,  $\gamma$  is the birth coefficient,  $Q_i$  is the set of APPs recognized by the  $i$ -th clonotype,  $Q_i = \|Q_i\|$ . We take identical parameters  $\mu, \gamma$  for all clonotypes as the results of numerical simulations with 10% random variations gave qualitatively the same results.

The TCC–APP recognition network is a random bipartite graph [13] of  $N$  TCC nodes connected to some of  $Q$  APP nodes with the probability  $p$  (Fig.1). Thus, on average, there are  $\nu = pN$  TCC competing for an APP, and  $\rho = pQ$  APPs recognized by a TCCs. Particular numbers follow binomial distributions with variances  $\sigma_\nu^2 = p(1-p)N$  and  $\sigma_\rho^2 = p(1-p)Q$ .

Demonstrate firstly the origin and stability of the stationary 'homeostatic' total size of the T-cell pool. Eq.(1) yields the closed linear first-order differential equation

$$\text{for the total number of T-cells } n_{\text{tot}} = \sum_{i=1}^N n_i: \quad (2)$$

$$\dot{n}_{\text{tot}} = -\mu n_{\text{tot}} + \gamma Q_{\text{rec}},$$

where  $Q_{\text{rec}}$  is the number of APPs that are recognized at least by one TCC<sup>2)</sup>. Combinatorial arguments give  $\langle Q_{\text{rec}} \rangle = [1 - (1-p)^N] Q$ . Eq.(2) has a unique stable equilibrium that gives the average T-cell repertoire size

$$\langle n_{\text{tot}}^* \rangle = \left\langle \frac{\gamma Q_{\text{rec}}}{\mu} \right\rangle = \frac{\gamma [1 - (1-p)^N] Q}{\mu}. \quad (3)$$

While the total number of T-cells exponentially fast converges to its stationary value (with the characteristic exponent  $\mu$ ), it does not tell anything about the behavior of each clonotype. Much can be learned, however, by constructing the absorbing region and the Lyapunov function analysis. Indeed, the right hand side of each line in (1) is negative when  $n_i > \gamma Q_i / \mu$ , which gives the upper boundaries of the absorbing region and guarantees the absence of infinite motion. The lower boundaries are  $n_i = \gamma Q_i^1 / \mu$ , where  $Q_i^1$  denotes the number of APPs recognized by  $n_i$  solely. It is easy to see that the clonotypes recognizing at least one such APP cannot get extinct.

The system (1) can be rewritten in the form

$$\dot{n}_i = -n_i \frac{\partial}{\partial n_i} V(n_1, \dots, n_N), \quad (4)$$

where

$$V(n_1, \dots, n_N) = \mu \sum_{i=1}^N n_i - \gamma \sum_{q=1}^Q \ln \sum_{i: q \in Q_i} n_i \quad (5)$$

is the generalized Lyapunov function. Calculating its time derivative one gets

$$\frac{dV}{dt} = \sum_{i=1}^N \frac{\partial V}{\partial n_i} \dot{n}_i = - \sum_{i=1}^N n_i \left( \frac{\partial V}{\partial n_i} \right)^2 \leq 0, \quad (6)$$

where the equality holds in the equilibria of (1) only. Otherwise  $V(n_1, \dots, n_N)$  is strictly decreasing in time, which excludes periodic or quasiperiodic motions.

Let us turn to numerical experiments to analyze the clonal size dynamics and statistics. The key process that we observe is the extinction of a substantial part of clonotypes. The distribution of clonotypes sizes is bimodal with peaks at zero and some non-trivial value (Fig.2). Increase in the graph connectivity with  $p$  leads to increase in the number of the extinct and the average size of the non-zero ones. It might seem that the classical competitive exclusion principle explains the effect [14]. Indeed, take  $N = 2$ ,  $Q = 2$  and assume that

<sup>2)</sup>It is straightforward to show that the stochastic variable  $n_{\text{tot}}$  is described by a Markovian birth and death process for which the mean, quasi-stationary distribution and other basic characteristics can be calculated.

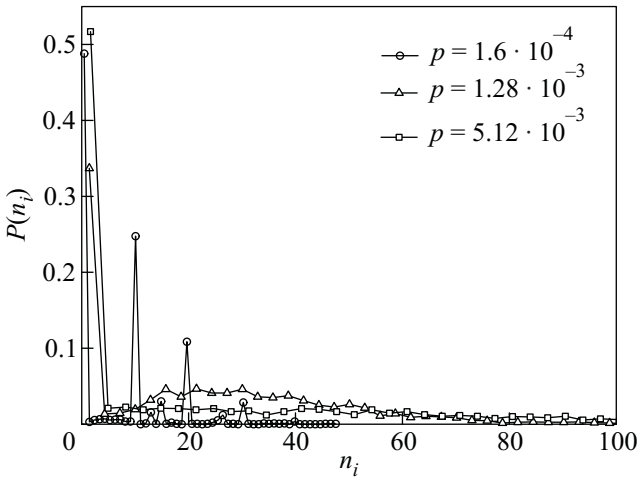


Fig.2. Limiting distributions of T-cell numbers in clonotypes for  $N = 2 \cdot 10^3$ ,  $Q = 5 \cdot 10^3$

the first clonotype recognizes both APPs but the second one does only one. Obviously,  $n_1 \geq n_2$  then, and the non-trivial equilibrium with  $n_1 \neq 0$  implies  $n_2 = 0$ . Combinatorial arguments, however, show that the probability of the APP set of the second clonotype being a subset of that of the first one gets exponentially small with  $Q$  and the explanation fails. Hence, the complex network structure underlies the phenomenon.

The change of variables  $\bar{n}_i = \frac{\mu}{\gamma} n_i$ ,  $\bar{t} = \mu t$  in (1) demonstrates that the only essential parameters are those characterizing the TCC-APP interaction network:  $p$ ,  $N$ ,  $Q$ . Thus, we fix  $\mu = 1$ ,  $\gamma = 10$  and analyze the extinction rate  $p_0 = p_0(p, N, Q)$  defined as the ratio of extinct clonotypes to their total number  $N$ . Numerical results demonstrate that the extinction rate reaches the minimum as  $p$  is increased from zero and  $N$ ,  $Q$  stay fixed (Fig.3). The probability that a TCC does not recognize a single APP and dies out in consequence is  $p_0 = (1-p)^Q \approx pQ$ . The probability that another clonotype will recognize the same APP is negligibly small if  $1 - (1-p)^{N-1} \approx p(N-1) \ll 1$  and competition between TCCs can be disregarded. The larger  $p$  the more chances are for a TCC to recognize at least a single APP but, at the same time, competition becomes more effective. The interplay leads to a minimum in  $p_0(p, N, Q)$  at the point well approximated by  $\nu = pN = 1$  when a single TCC recognizes a given APP on average. The depth of the minimum depends on  $Q/N$ : when APPs are in large excess almost no extinction is observed, and when near parity or in shortage the competition becomes strong before the APP recognition gets sufficiently diverse.

Equivalently remarkable are the results in the  $p \approx 1$  domain. It is easy to see that  $p_0(1, N, Q) = 0$  as each TCC would recognize all APPs and no one would have

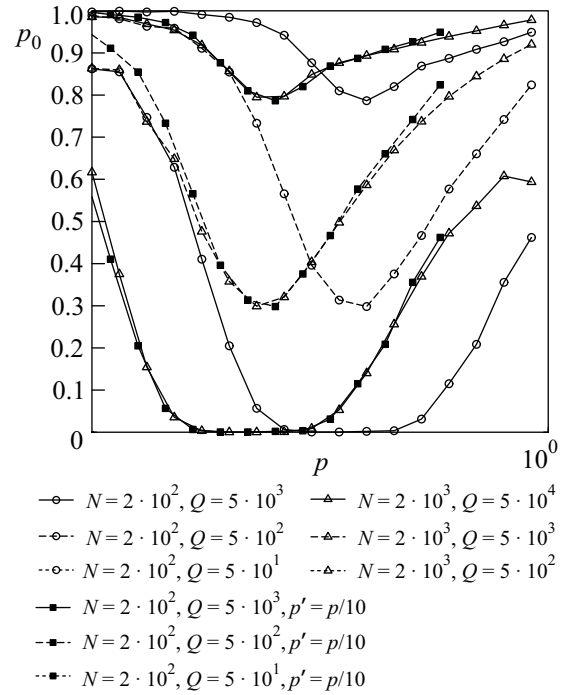


Fig.3. Dependence and scaling of the clonotype extinction rate  $p_0$  on the number of T-cell clonotypes  $N$ , APPs  $Q$ , and recognition probability  $p$

an advantage. It follows that  $p_0(p, N, Q)$  must have a maximum between the minimum and  $p = 1$ . Approximating the behavior near  $p = 1$  one can argue that those TCC that do not recognize at least one APP while at least one TCC recognizes all will die out with the the correspondent probability  $1 - p^Q \approx (1-p)Q$ . When the probability that none of TCCs will recognize all APPs  $(1-p^Q)^N$  becomes substantial competitive exclusion arguments fail and the complex network structure governs the competition.

As the statistical properties of large bipartite networks depend on intensive parameters  $\nu$  and  $\rho$  only, we have hypothesized that the extinction rate should (approximately) do the same. Plotting the curves for ten times smaller  $N$  and  $Q$  we observe a strikingly similar behavior and downscaling abscissas by ten we get a very good coincidence indeed (Fig.3).

This scaling is a cornerstone for linking the model to physiological parameters. Experimental data estimate the total number of T-cells as  $\langle n_{tot} \rangle = 10^{11} - 10^{12}$  and the number of clonotypes as  $N = 10^7 - 10^8$  in healthy adult humans [3]. For definiteness we take  $n_{tot} = 10^{11}$ ,  $N = 10^8$ . Estimates for  $\mu^{-1}$  vary from several weeks to years [15]. We take  $\mu^{-1} = 1$  year for simplicity and rescaling to other values will be straightforward. The main type of the antigen presenting cells are den-



creases transients. Remarkably, the characteristic transient time remains essentially the same if only constant  $\rho$  is maintained and  $\nu$  varies. Hence, the dependence upon  $N$  is weak if any. These properties are in marked contrast with the 'rules of thumb' in collective dynamics of large ensembles: normally, the more coupling between the nodes the faster transient processes end up [13]. The more nodes there are the longer transients should take, but not in this case again.

Let us get a deeper insight into the phenomenon. Plotting the time dependence of the TCCs sizes one immediately notices that the competition process bears pronounced features of sequential activity (Fig.6). Extinction does not occur in parallel. On the contrary,

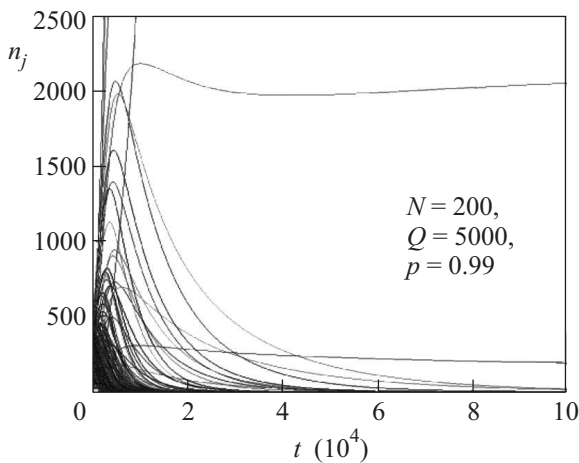


Fig.6. Examples of transient competition in large TCC networks. Note initial proliferation of the clonotypes that eventually go extinct for a class of initial conditions

the weakest TCCs die first and during that time the stronger ones (also destined to extinction) increase their size. Only after their 'victims' get extinct the stronger ones start effective competition with each other, when the strongest will survive. Therefore, the sequential transient competition leads to the TCCs sizes distribution drastically different from the final stationary state.

Summarizing, we have studied the competition for survival signals in large many-species – many-sources random bipartite networks modeling the evolution of naive T-cell repertoire. The exponential convergence to the stationary size of the T-cell pool was demonstrated, as well as the absence of periodic or chaotic oscillations. The exponentially fast extinction of clonotypes has been observed and identified as a mechanism for selection of the most efficient clonotypes and improving diversity of the repertoire. The extinction rate was shown to be a bimodal function of the TCC-APP recognition probability with the absolute maximum (at zero) and minimum

(at one) additionally. This function obeys an approximate scaling with the size of the connectivity network if the average number of competitors for a single APP and that of TCCs recognizing a given APP remain constant.

The transient process to the stationary state has turned out to be a prolonged sequential process of competition with the approximately linear scaling of characteristic time with the total number of APPs. It was demonstrated that the distribution of clonal sizes during the transient is substantially different from that in the final stationary state.

Having calibrated the model to the physiologically plausible parameters we derived for the first time the estimates of physiological values of the T-cell receptor – APP recognition probability  $p \approx 1.5 \cdot 10^{-7}$  and T-cell clonotypes niche overlap  $\nu \approx 15$ . We have discovered that the transient times may correspond to the life time scale. We hypothesize that the T-cell homeostasis is, in fact, a complex transient process manifested in significant changes in the TCCs sizes (while the total size of the repertoire stays stationary) in healthy adults additively to the renewal from thymus. Other mechanisms of the T-cell regulation not incorporated in the model are expected to add complexity to the dynamics rather than simplify it. The discovered type of sequential transient dynamics advances our understanding of competition in complex networks as the only previously known prerequisite has been the existence of stable heteroclinic sequences [17]. These principles are expected to apply to competition in other multi-cellular networks and population ecology as well.

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