# Some thoughts on the ontogenesis in B-cell immune networks

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**Abstract.**

We are interested in modeling theoretical immunology within a statistical mechanics flavor: focusing on the antigen-independent maturation process of B-cells, in this paper we try to revise the problem of self vs non-self discrimination by mature B lymphocytes. We consider only B lymphocytes: despite this is of course an oversimplification, however such a toy model may help to highlight features of their interactions otherwise shadowed by main driven mechanisms due to i.e. helper T-cell signalling.
By analyzing possible influences of the ontogenesis of the immune system on the final behavior of B lymphocytes, we try to merge over the purely negative selection mechanism at their birth with the adult self-regulation process. The final goal is a "thermodynamical picture" by which both the scenarios can exist and, actually, be synergically complementary: Trough numerical simulations we impose on a recent scheme for B-cell interactions, that part of self-reactive lymphocytes are killed during the ontogenesis by which two observations stem: At first the so built system is able to show anergy with respect to the previously encountered self even in its mature life, then this naturally leads to an increasing variance (and average) in the connectivity distribution of the resulting idiotypic network. As a consequence, following Varela perspective, this shift may contribute to push to anergy those self-directed cells which are free to explore the body: identifying the latter as the highly connected ones, anergy is imposed even via the B-network regulation, and its strength is influenced by the negative selection.

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# 1 Introduction

Immunology is probably one of the fields of science which is experiencing the greatest amount of discoveries in these decades: As the amount of works increases, the need for minimal models able to offer a general, coarse grained, framework where these may find a collocation is a must for modelers interested in this field.
Despite actors in the immune system are many, for the sake of simplicity, we are going to focus only on the B-cell ensemble and, for the sake of clearness, we allow ourselves in presenting a streamlined introduction to the main concepts on their world and a state of the art in self/non-self discrimination, on which we will rely soon.
The purpose of the immune system is to detect and neutralize the molecules, or cells, dangerous for the body (antigens, which could be foreign invaders - e.g. viruses or bacteria - or deranged - e.g. cancerous - cells of the host), without damaging healthy cells [1]. The humoral response performed by B lymphocytes consists in analyzing the antigen by each family of identical B-cells (clones), then the one/s with the best matching antibody[4] undergoes clonal expansion and releases its immunoglobulins (clonal selection theory): the latter are able to bind the pathogens and neutralize their chemical abilities; then, the resulting complex is destroyed by macrophages and order established again.
For achieving this goal, the immune system needs an enormous number of different clones, each one having a particular receptor for antigens. As these receptors are generated randomly by somatic mutation at the genetic level, the body may produce both antibodies attaching to (a part of the) intruders (i.e. viruses), as well as to internal ones (self reactive lymphocytes), which, if not carefully checked, may induce autoimmunity, an obviously unwanted feature.
To avoid this failure, at least two mechanisms are thought to work (for self/non-self discrimination), at different levels in the immune systems as we are going to resume.
B-cells are generated, and maturate, in the bone marrow, where they are exposed to "negative selection rule"[5]: In a nutshell, driven by the nurse-cells, these lymphocytes are made to interact with an (available) repertoire of self-antigens, namely molecules/cells belonging to the host body, and those who are found to respond to them (so potential autoimmune B-cells) experience induced apoptosis, such that only B-cells unable to attach to the available self survive and share the freedom of exploring the body thereafter [1].
It is in fact widely accepted that the bone marrow produces daily $\sim 10^7$ B cells, but only $\sim 10^6$ are allowed to circulate trough the body, the remaining 90% undergoing apoptosis because targeted as self-reactive [32]: as shown for instance by Nemazee and Burki [23], this depletion of the potential defense is due to the negative selection (clonal deletion) of immature B-cells expressing self-reactive antibodies. However as only a fraction of self-antigens are present into the bone marrow, self-reactive lymphocytes not expressing specific receptors (BCR) against the available self are allowed to circulate freely by this first security procedure: another mechanism must act at peripherals levels (i.e. in the lymphonodes, spleen and lever).
Coherently, after their experiments, Goodnow was been able to show [13] that these self-reactive lymphocytes indeed exist in the body, but instead of undergoing apoptosis, they experience anergy in their responses (namely, under the proper stimulus, they do not responde). Furthermore his experiments

[4]The first postulate of immunology states that, hypersomatic mutation apart [16, 29], a given clone of B-cells -namely a family of identical lymphocytes- produces always the same antibody.

[5]We only stress here strong differences among B-cell maturation in the bone marrow and T-cell one in the thymus, due to the lacking of TCR by the humoral effectors [12][21][22]. Unlike TCR that evolved to recognize characteristic patterns of pathogens, BCR on B-cells is primarily diversified in random fashion and has not evolved to recognize a particular structure. Therefore each B cell can not discriminate self versus non self alone [18].

showed that this anergy could be related to the corresponding low expression of IgM on the external membrane of the self-reactive lymphocytes (establishing a ratio $1:20$ with an ordinary one) implicitly suggesting both an effect in their response function by the network[6], as well as that a reduction in the expression level of surface receptors IgM quantitatively resulted via a biased signal transduction producing the anergic state.
Furthermore, still highlighting the need for a second pathway of control, experimentally, in a healthy body, a low dose of (some of) self-directed antibodies is commonly found (negative selection is not exhaustive), and theoretically (highlighting the importance of the B-cell network alone), if the amount of information needed to tackle a response (i.e. the amounts of epitopes) is believed to range in the order $\sim 10^3$[25], within a pure action-reaction approach, the immune system would need $O(2^{10^3})$ different clones, which is an enormous number with respect to the amount of actual ones found in the body (i.e. $\sim O(10^{10})$ [25]): information for pattern recognition must be spread over a network of interacting B-cells.
So, self reacting clones that have not been eliminated in the bone marrow become unresponsive to (self)-antigen, which is termed "anergy": In these cells, continued binding of self-antigen is required to be kept in anergic state [11][14].
Following Kitamura [18], a key in understanding the strategy by which B-cells manage self/non-self discrimination (despite the very incomplete knowledge of the BCR signaling pathways) is their double signalling activation need (namely the presence of the antigen and the stimulation by the cognate T helper): while the double signaling induces clonal expansion, only one signal (the [self]-antigen) may induce a suppression. As a consequence, B-cell network may synergically use the helper T-cells both for activation against pathogens as well as for anergy induction with respect to self ones (at T-helpers are equipped by TCR).
Furthermore, the anergic B cell shows several features that characterize its "peculiarity": cell surface expression level of BCR (IgM) is reduced and that of $CD5$ (IgD) increased, lifespan is shortened and entry into the lymphoid follicles is prohibited[7]. Their BCR are desensitized and therefore the B cell do not proliferate in response to antigens even in the (not usual) presence of cognate T-cell help (which implicitly may suggest other mechanisms then the need of double signaling by antigen and helper alone) but are instead made anergic or eliminated by Fas-induced apoptosis (AICD).
Despite this may actually be the main strand for explaining self/non-self discrimination, other mechanisms may cooperate, and, among these, as experiments in vitro with the Jerne network are prohibitive by construction, we plan to investigate them trough statistical mechanics simulations: the goal is to isolate this possible path from the main one and see if it can contribute to the overall regulation.
As a consequence we spend a few words on this network:
The idea of this internal B-core network appeared early in immunology [8], and its concretization happened when Jerne [17], in the 70's, suggested that each antibody must have several idiotopes which are detected by other antibodies. Via this mechanism, an effective structure of interacting antibodies is formed, in which the latter not only detect antigens, but also function as individual internal images of them and are themselves detected and acted upon. These network interactions provide a "dynamical memory" for the immune system, by keeping the concentrations of antibodies (especially those representing encountered antigens) at appropriate levels. This can be understood as follows: At a given time a virus is introduced in the body and starts replication. At high enough concentration, it is found by the proper B-lymphocyte counterpart: let us consider, for simplicity, a virus as a string of information

[6]The term "network" here is meant to include the whole immune system interactions, not just the B-core, so at first exchanges among B-cells and T-cells, cytokine messengers and so on [1].

[7]This suggests that anergic B cells, in contrast to non-self reactive B cells, fail to compete for survival of chemotactic factor [6].

(i.e. 1001001) [8]. The complementary B-cell producing the antibody Ig1, which can be thought of as the string 0110110 then will start a clonal expansion and will release high levels of Ig1. As a consequence, after a while, another B-cell will meet 0110110 and, as this string never (macroscopically) existed before, attacks it by releasing the complementary string 1001001, that, actually, is a "copy" (internal image) of the original virus but with no DNA or RNA charge inside [9]. The interplay among these helps in keeping memory of the past infections.
Once a network theory is achieved, it is easy to understand that, given the "hyper-fine" recognition mechanism, this implies the connectivity of such a network to range over several orders of magnitude: as a result a biological interpretation is handily and originally due to Varela and Coutinho [19, 28, 31]: nodes (i.e. clones) which are poorly connected are thought of as antigen-directed as they can easy respond to external fields (roughly speaking are more approximable as single particles), while nodes that are highly connected can probably be self-directed as they can be strongly influenced by the (large amount of) nearest neighbors, which, in this case, may keep them in a state of anergy.

## 2 The minimal model

Focusing on these "emergent properties" of B-cell networks, (and neglecting investigation during B-cell's birth trough the negative selection), inspired by pioneering ideas of thermodynamical flavor [24] in a recent series of papers [3, 4, 2] a statistical mechanics model for such systems has been introduced. Within that framework the distinction among self and non-self was thought of at a cooperative level alone, in a pure Varela style: no ontogenesis were investigated and no learning rules discussed, while it was shown how to obtain a scale free weighted connectivity distribution from a wide class of antibody's interactions, as a benchmark for self-non self discrimination a posteriori.
Despite a scenario able to recover several real features of the immune system was already achieved in this way, however, a complete elimination of a learning process during the ontogenesis was unrealistic [12] and indeed its existence could alter the mature network functionalities: so we want to move over and show that the two pictures discussed in the introduction (negative selection and idiotypic network regulation) may act synergically and naturally accounted by the model itself.
Taking an antibody as a binary vector made up of the possible expression of $L$ idiotopes, we assume that these can be thought of as strings of the same length[10], such that, as the elementary $L$ idiotopes can be introduced as,

$$\xi_1 = (1,0,0,...,0),\ \xi_2 = (0,1,0,...,0),\ ...,\ \xi_L = (0,0,0,...,1), \tag{2.1}$$

forming an orthogonal base in the $L$-dimensional space $\Upsilon$ of the antibodies, in such a way that the generic $i^{th}$ antibody $\xi^i$ can then be written as a linear combination of these eigenvectors $\{\xi^i\} = \lambda^i_1\xi_1, \lambda^i_2\xi_2, ..., \lambda^i_L\xi_L$, with $\lambda^i_\mu \in (0,1)$ accounting for the expression (1) of a particular $\mu$th idiotope or its lacking (0).
In this way, as often done in modern modeling of antibody affinities [26][27], we relax the earlier simplifying assumption of "a perfect mirror of a mirror" for the interacting immunoglobulins simply asking

[8] The dichotomy of a binary alphabet in strings mirrors the one of the electromagnetic field governing chemical bonds.
[9] This counter-images have been revealed experimentally in several researches, i.e. [5].
[10] The molecular weight for each Igs is accurately close to $15 \cdot 10^4$ and each idiotope on average is large as each other (see [15])

that the better the matches among idiotopes, the stronger the stimulus occurring between the respective clones via these messengers.

Moreover, the system is made up of an ensemble of $N$ different clones, each composed of $M$ identical lymphocytes; a given lymphocyte $i$ (whose corresponding antibody is $\xi_i$), is then described by the dichotomic variable $\sigma_i^\alpha = \pm 1$, with $\alpha = 1, ..., M$, and $i = 1, ..., N$, such that the value $-1$ denotes an anergic/absent state (low level of antibodies secretion) while the value $+1$ a firing state (high level of antibodies secretion).
To check immune responses we need to introduce the $N$ order parameters $m_i$ as local magnetizations:

$$m_i(t) = \frac{1}{M} \sum_{\alpha=1}^{M} \sigma_i^\alpha(t). \tag{2.2}$$

From the magnetizations $m_i \in [-1, 1]$, which play the role of the principal order parameters, we can define the concentrations of the firing lymphocytes belonging to the $i^{th}$ family as [11]:

$$c_i(t) \equiv \exp\left[\tau \frac{(m_i(t)+1)}{2}\right], \quad \tau = \log M. \tag{2.3}$$

Further we introduce the Hamiltonian $H$ which encodes the interactions among lymphocytes as well as the interactions among lymphocytes and the external antigens:

$$H = H_1 + H_2 = -N^{-1} \sum_{i<j}^{N,N} J_{ij} m_i m_j - c \sum_{k}^{N} h_k m_k, \tag{2.4}$$

where $c$ rules the amount of the external antigen present in the host and $h$ its epitopal characteristics, whose links with the antibodies will be discussed hereafter:
In fact, we must briefly resume how the interaction matrix $J_{ij}$ is built up (and consequently $h_k$): Given two strings $\xi_i$ and $\xi_j$, their $\mu$-th entries are said to be complementary, iff $\xi_i^\mu \neq \xi_j^\mu$. As each entry $\mu$ of the $i$-th string (Ig) is extracted randomly according to the discrete uniform distribution in such a way that $\xi_i^\mu = 1$ ($\xi_i^\mu = 0$) with probability $1/2$, given a couple of clones, say $i$ and $j$, therefore the number of complementary entries $c_{ij} \in [0, L]$ can be written as

$$c_{ij} = \sum_{\mu=1}^{L} [\xi_i^\mu (1 - \xi_j^\mu) + \xi_j^\mu (1 - \xi_i^\mu)]. \tag{2.5}$$

The affinity between two antibodies is expected to depend on how much complementary their structures are. In fact, the non-covalent forces acting among antibodies depend on the geometry, on the charge distribution and on hydrophilic-hydrophobic effects which give rise to an attractive (repulsive) interaction for any complementary (non-complementary) match. Consequently, we assume that each complementary / non-complementary entry yields an attractive / repulsive contribute. In general, attractive and repulsive contributes can have different intensity and we quantify their ratio with a parameter $\alpha \in \mathbb{R}^+$. Hence, we introduce the functional $f_{\alpha,L} : \Upsilon \times \Upsilon \to \mathbb{R}$ as

$$f_{\alpha,L}(\xi_i, \xi_j) \equiv [\alpha c_{ij} - (L - c_{ij})], \tag{2.6}$$

[11] The bridge among concentrations in chemical kinetics and statistical mechanics has been early investigated by Thompson [30] in the context of red cells, but the same should hold even for the white ones and, however, does not affect our investigation.

which provides a simple measure of how "affine" $\xi_i$ and $\xi_j$ are. In principle, $f_{\alpha,L}(\xi_i, \xi_j)$ can range from $-L$ (when $\xi_i = \xi_j$) to $\alpha L$ (when all entries are complementary, i.e. $\xi_i = \bar{\xi}_j$). Now, when the repulsive contribute prevails, that is $f_{\alpha,L} < 0$, the two antibodies do not match each other and the coupling among the corresponding lymphocytes $J_{ij}(\alpha, L)$ is set equal to zero, conversely, we take $J_{ij}(\alpha, L) = \exp[f_{\alpha,L}(\xi_i, \xi_j)]/\langle \tilde{J} \rangle_{\alpha,L}$, being $\langle \tilde{J} \rangle_{\alpha,L}$ the proper normalizing factor so to keep unitary the average coupling.
Hence, nodes can interact pairwise according to a coupling $J_{ij}(\alpha, L)$, which is defined as:

$$J_{ij}(\alpha, L) \equiv \Theta(f_{\alpha,L}(\xi_i, \xi_j)) \frac{\exp[f_{\alpha,L}(\xi_i, \xi_j)]}{\langle \tilde{J} \rangle_{\alpha,L}}, \tag{2.7}$$

where $\Theta(x)$ is the Heaviside function returning 1 if $x > 0$, and 0 if $x \leq 0$; notice that the affinity matrix is symmetric, namely $J_{ij}(\alpha, L) = J_{ji}(\alpha, L)$ [12]. The coupling $h_i^k$ between the antigen $\bar{\xi}_i$ and the antibody $\xi_k$ is defined analogously.
From a statistical mechanics perspective, the Hamiltonian is the average of the "energy" inside the system and thermodynamic prescription is that the system tries to minimize it. As a consequence, according to $H_1$, increasing the antigen concentration makes the antibody response grow such that if $c(t_2) > c(t_1)$, with $t_2 > t_1$, the same happens for each involved clone $m(t_2) > m(t_1)$ and viceversa. Moreover, according to $H_2$, two generic clones $i$ and $j$ in mutual interactions, assuming here $J_{ij} > 0$, tend to imitate one another, i.e. if $i$ is quiescent, it tries to make $j$ quiescent as well (suppression), while if the former is firing it tries to make firing even the latter (stimulation), and symmetrically $j$ acts on $i$. At this stage we deal with a mature immune system whose order parameters, namely the magnetizations, are centered symmetrically distributed. Despite agreement with phenomenology [3][4][2], thinking that the absence of a stimulus can be understood as a stimulus, seems difficult to be explained and surely not exhausted here [13]. However if we write down the internal field acting on the generic $i^{th}$ clone, calling it $\varphi_i$ and labeling the weighted connectivity of such a node as $w_i = \sum_j J_{ij}$, we see that

$$\varphi_i = \sum_j J_{ij} m_j + h_i,$$

where we meant the antigen with $h_i$ (and set it to zero now for clearness, namely $h_i = 0$). If we now switch to the concentrations (through eq. 2.3), we see that this field can be written as

$$\varphi_i = \frac{2}{\tau} \sum_j J_{ij} \log c_j - w_i.$$

From a B-cell concentration viewpoint each clone experiences in this way a contribution from the weighted connectivity of the idiotypic network that pushes to anergy, furthermore, the larger the connectivity of the node, the stronger the resulting imposition to anergy[14].

[12] We stress that, built in this way the affinity matrix, we can construct a dynamics respecting detailed balance [30], as a result relaxation to Maxwell-Boltzmann distribution in ensured an we can use standard MonteCarlo techniques in simulations.

[13] We remember however that "similar" mechanisms initially difficult to be understood have already appeared several times in the scientific literature, i.e. the paradigmatic negative solutions of the Dirac equation led to the concept of lacunaes in quantum mechanics.

[14] Low connectivity inhibition experienced by the non-self directed clones accounting for the low dose tolerance phenomenon [1][24].

# 3 Ontogenesis

As we mentioned, during the ontogenesis of the repertoire of B-cells, those interacting with self-antigens undergo to negative selection (roughly speaking are killed[15]). As a consequence, here we implemented the following learning rule: At the beginning, and once for all, $N_S$ vectors coding for their corresponding antigens (randomly drawn from a uniform distribution) are arbitrarily labeled as "self" and stored into the algorithm.
Then, each creation time[16] a set of $P < N$ newborn lymphocytes is generated and each of these $P$ B-cells is made to interact with all the $N_S$ self-antigens: those who are able to bind this available self (namely, display a positive affinity) are killed.
As a consequence only a fraction $P^* < P$ is retained. Then another set of $P < N$ of newborn lymphocytes is randomly created and the whole ensemble of $P + P^*$ of lymphocytes is made to interact with the $N_S$ self-antigens. Again those able to bind the self are eliminated. The process stops when a size-desirable ensemble of lymphocytes is created (i.e. a repertoire of size $N$).
Once this ontogenetic process finishes, we study the property of the network obtained in this way and compare them with respect to a network resulting in a purely random fashion without any learning rule.

## 3.1 Numerical implementation

To test the features of a so generate artificial immune network, we use Monte Carlo simulation: Following a standard statistical mechanics approach [7] the dynamics can be written as

$$\sigma_i^\alpha(t+1) = sign\left(\tanh(\beta\varphi_i(t)) + \eta_i^\alpha(t)\right), \tag{3.1}$$

where $\varphi_i(t)$ is the overall stimulus felt by the $\alpha$ lymphocyte of the $i$ clone , namely

$$\varphi_i(t) = N^{-1}\sum_j^N J_{ij}m_j(t) + h_i(t), \tag{3.2}$$

and the randomness is in the noise implemented via the random numbers $\eta_i^\alpha$, uniformly drawn over the set $[-1, +1]$. The impact of this noise on the state $\sigma_i^\alpha(t+1)$ is tuned by $\beta$, such that for $\beta = \infty$ the process is completely deterministic while for $\beta = 0$ it is completely random.
As the affinity matrix is symmetric, for this detailed balanced system, the sequential stochastic process (3.1) can be implemented on a machine via Glauber dynamics, with the following expression for the transition rate $W_i$

$$W_i(\sigma_i^\alpha) = \Big(1 + \exp(\beta\Delta H(\sigma_i^\alpha;\xi))\Big)^{-1}, \tag{3.3}$$

where $\Delta H(\sigma_i^\alpha;\xi) = H(F_i^\alpha\sigma_i^\alpha;\xi) - H(\sigma_i^\alpha;\xi)$ and $F_i^\alpha$ is the "spin-flip" operator that reverses $\sigma_i^\alpha \to -\sigma_i^\alpha$.
Using these probability rates, it is immediate to define a Monte Carlo scheme (MC) for simulating

[15]The biological motivation of this initialization is that B-cell antigen receptor signal transduction machinery transiently activate the cell but rapidly endocytosis any antigen that bind BCR to ensure the cessation of the initial activation of the cell. Immature B-cells (during ontogenesis) are not yet equipped for the antigen presentation, furthermore, no helper T-cells are available to double signaling the activation process, the whole pushing the cell to induced apoptosis.

[16]The genesis of B-cells should be a continuous time process in the bone marrow, however, dealing with numerical simulations, we discretize the time such that each time iteration a -fixed- amount of lymphocytes is generated.

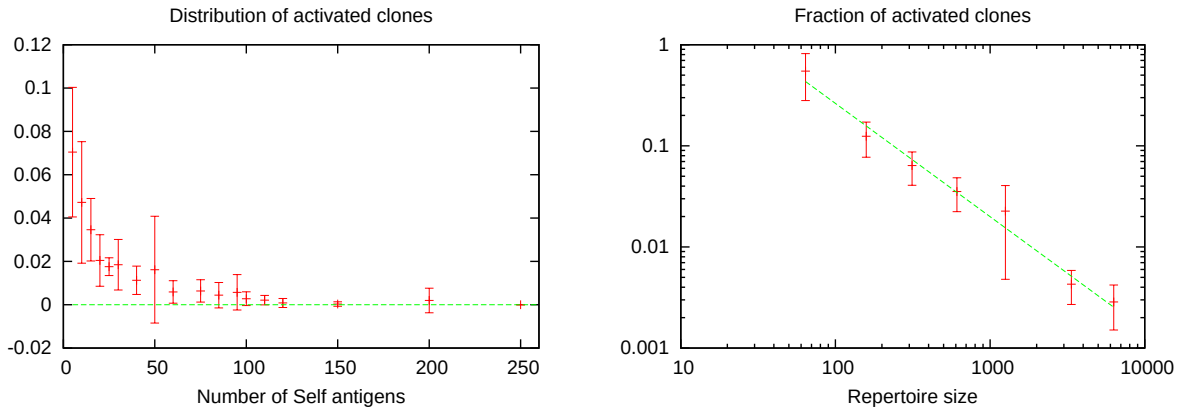


Figure 3.1: Left: Distribution of the activated clones for an immune network at rest built up by $N = 628$ clones versus the amount of self-antigens used to generate the repertoire with antibodies made of by strings of $L = 11$ epitopes. Right: Finite size scaling of the system. Averaged response of the network created trough a repertoire with $L = 8, ..., 14$ epitopes (keeping the fractions of the present clones and self-antigens constant) against one (randomly chosen) antigen of the repertoire itself. Coherently with the request that only a finite fraction of clones remains active increasing the network size, the fit is obtained trough $O(N^{-1})$ power (the exact value of the fit with $N^x$ gives $x \sim -1.12$).

the network: The general system setup is made of by $N, N_S, N_A$ elements, where $N$ is the amount of the (mature) repertoire, $N_S$ the amount of learned self-antigens and $N_A$ the amount of available external antigens. We study the response of the system against the global field composed always by $N_S$ self antigens and a variable amount from the $N_A$ ensemble. The chosen dynamics is the standard Metropolis where each MC iteration is built by $N \cdot M$ steps (i.e. the amount of cellular automata in the system): for each of these steps one lymphocyte $\sigma_i^\alpha$, randomly drawn over the repertoire, is chosen and flipped $\sigma_i^\alpha \to -\sigma_i^\alpha$: the variation into the energy term $\Delta H(\sigma; \xi)$ is then evaluated and if this delta is negative such a trial move is retained by the system, otherwise randomly rejected with probability $\propto \exp(-\beta H(\sigma; \xi))$.
The value of $\alpha$ is kept to $\alpha = 0.7$ following biological matching as explained in [3].

## 3.2 Results: Self-tolerance, memory and saturation.

At first we stress that, as simulations with a realistic amount of clones are still too heavy in CPU time consuming, we worked at various repertoire sizes but we tested the robustness of the results trough a finite size scaling which is reported in Fig.3.1 (right).
Once the repertoire has been created (and an established network of interacting B-cells achieved), external antigens are presented to it and responses are checked. At first, since we constructed the repertoire with the intent of tolerance to self-antigens, we check its robustness by presenting to the system a field composed only by self-antigens: at low temperature, anergy to self is completely fulfilled (not shown in plots), for each experienced field made of by $1, .., N_S$ self-antigens. Furthermore, as shown in Fig.(3.1) (left), we notice that higher the number of self-antigens $N_S$ stored by the system (at fixed repertoire size $N$), sharper its epitopal matching in binding antigens as only a small amount of highly affine clones are

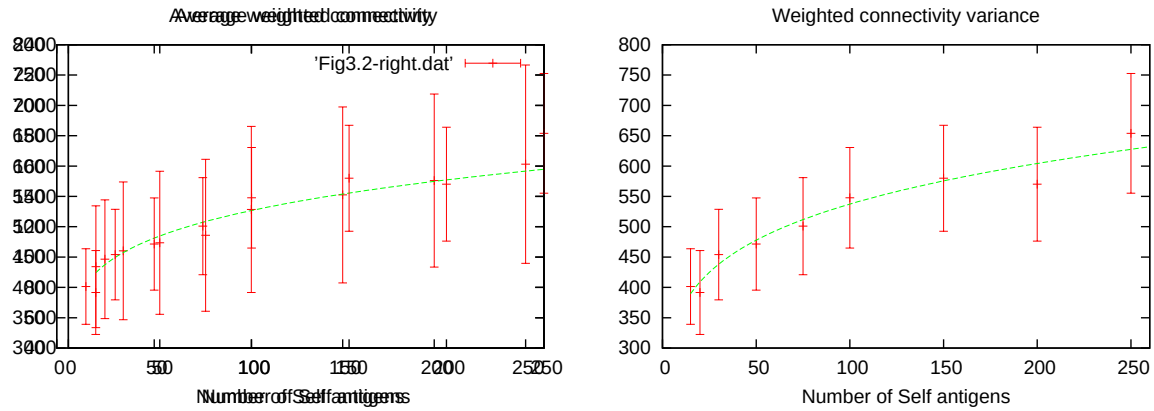


Figure 3.2: Left: Averaged weighted connectivity for different repertoires generated increasing the size of the experienced self $N_S$ at ontogenesis. Right: Standard deviation of the averaged connectivity plotted at left. We stress that both these quantities increases with $N_S$, suggesting relations among learning in ontogenesis and improved performances in mature behavior. The power-laws $N_S^W, N_S^{\sigma_W}$ obtained by the fits respectively for the connectivity and its variance gave $W = 0.19 \pm 0.02, \sigma_W = 0.17 \pm 0.02$.

available to responde. This can be a cross feature of the ontogenesis: a direct cause is the generation of holes in the repertoire, such that, the larger the hole/s the harder the ability in binding; however, there is another feature ongoing: both the average weighed connectivity and its variance become higher as $N_S$ increases (see Fig. (3.2) left and right); the whole highlighting a non trivial effect of this learning process into the mature clonal network: As $N_S$ grows both these quantities grow[17]. This growth with $N_S$ can be a very important point because, with respect to a standard random network with no learning process (i.e. $N_S = 0$), this system displays a larger variance of a (larger) averaged weighted connectivity: From Varela perspective [28, 31] this allows a better response of the mature B-cells against the antigens and a stronger anergy for those clones self-directed.
Coherently with the previous picture of this artificial immune system, we note that as $N_S$ gets bigger the amount of responding clones get smaller since, as their average connectivity is increasing, their responses become weaker, in perfect agreement with the Varela picture.
Another interesting observed result is the idiotypic nature of such a network (not shown): as the responding clones become activated, they induce activation to the ones with higher complementary to them (Jerne images of the antigens) and, while this activation does not propagate extensively trough the system (only dimers and four loops are observed with our system sizes), these images actually are found to participate in keeping memory of the antigen, once it is removed.
To check abilities in storing memories of the past infections in this artificial immune network, keeping in mind that trough MC simulations we only access equilibrium information, we collect snapshots of the system and confront those representing it immediately after the antigen infection (when all the responding clones and their idiotypic counterparts are activated) and the ones representing the system when the antigen has been removed and the network stabilized again. As shown in Fig. (3.3) (for two different examples, i.e. exposures to one (left) or five (right) antigens simultaneously), the system maintains (the proper) clones activated even after the explicit presence of the stimuli has been removed.
Of course the resulting antibody (or lymphocyte) concentrations are lower with respect to the first re-

[17]The similar behavior of the growth process among average and variance should not surprise as these networks are random and not too far from the simplest Erdos-Renyi ones [3], i.e. Poissonian.

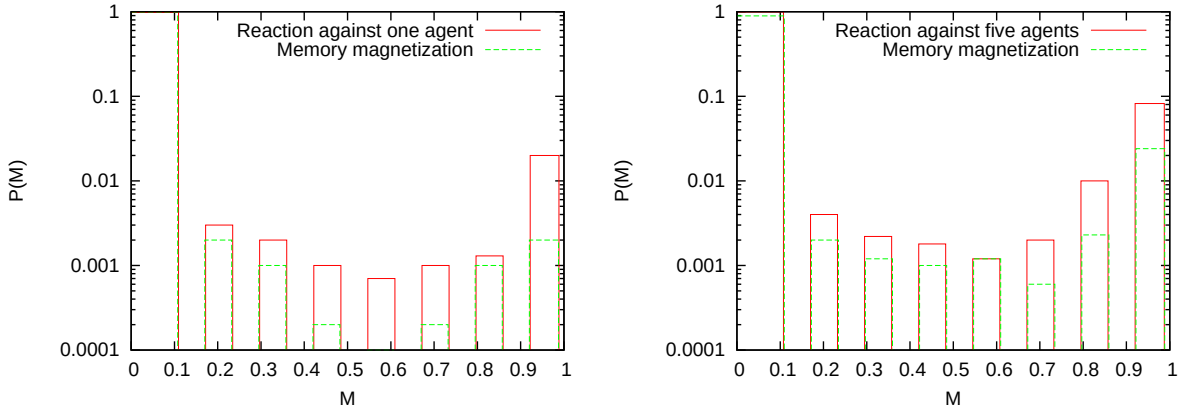


Figure 3.3: Left: Distribution of the magnetizations during one antigen attack (red) and after its removal and the successive network equilibration (green). Right: Distribution of the magnetization during five contemporary attacks (red) -by different antigens- and after their removal and the successive network equilibration (green). The examples show a system made of by 1257 clones and 20 self-antigens.

sponse as these are thought of as only memory cells (bridging what in physics is called as remanent magnetization, an hysteresis effect[18]). However, despite the system is extremely able to respond sharply to the desired infection (or trough a proper best fitting antibody or trough a linear combination of the "enough matching" ones from the repertoire, due to hysteresis (which are unavoidable features as they ensure the dynamical memory), infection after infection, the system starts behaving plastically and eventually, after a certain threshold unwanted activations may appear (reflecting senile autoimmunity) and if this iteration continues, it stops working at all (no recognition is possible any longer).
The fraction of the activated clones as a function of the different antigens continuously experienced is reported in Fig.(3.4):
This percolation activation can be easily understood from the perspective of spin glasses (as well as its aging properties previously discussed) due to its strong analogy to a diluted random field model into a magnetic field: since the system works at low temperature, it undergoes a first order phase transition for a critical value of the external field [20][10].

# 4 Discussion

In this paper we investigated the ability of memorizing -and its consequences- for a model of B-cell interactions only. The main goal was a satisfactory picture by which negative selection mechanism at ontogenesis may act and operate synergically with the idiotypic network regulation (for the mature system) in self/non-self discrimination.
It is worth noticing that key mechanisms, as helper double signalling, are fundamental for a complete discrimination process, however, we investigated this "sub-shell" of the system alone to highlight features

[18]In this model there is no a-priori difference among plasma and memory B-cells; the latter are simply though of as remanent magnetizations, such that, once the antigen is removed -say $h_k = 0$-, its corresponding magnetization $m_k \geq 0$, using the hysteresis as a generator of dynamical memory trough the network.

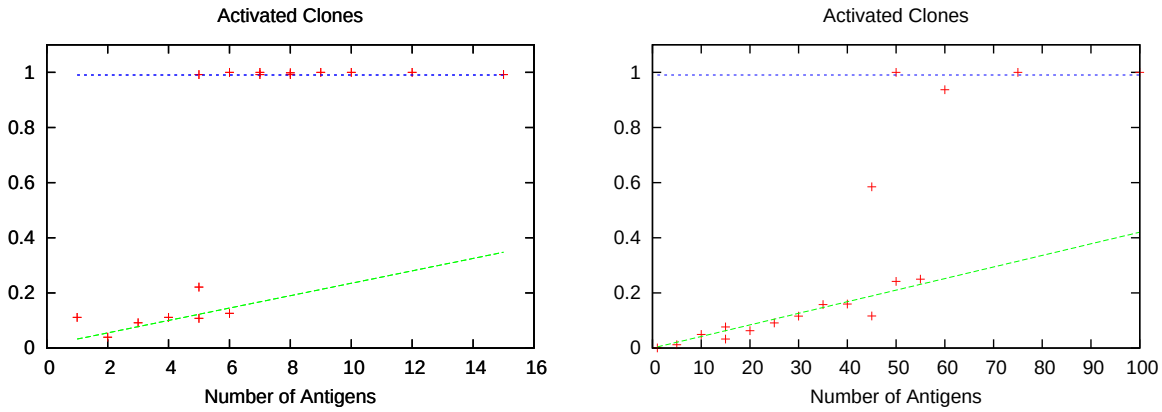


Figure 3.4: Fraction of the activated clones as a function of the antigens presented to the system. Left: The system is made of by 611 clones and 10 self-antigens. Right: The system is made of by 3352 clones and 50 self-antigens.

which can result purely by its actions and which can be more difficult to be revealed when looking at the system as a whole. As a result our goal is not meant as an explanation of the main strand in self/non-self discrimination, but an investigation of the mechanisms which can participate stemming from the network perspective.
In fact, from one side it is now widely accepted the key role of T helpers in such a regulation: silencing of self-reactive B cells must be initiated by the binding of self-antigens and because a B-cell alone cannot distinguish between self and non-self, the decision to become anergic must be based on whether secondary signals are received within a specific time frame: if not, the cell undergoes BCR desensitization, which ultimately results in anergy. However, as biological systems, for structural stability, rarely allow only a single pattern of realization of a macroscopic behavior, we decided to isolate, in numerical simulations, the B-cell network and investigate the relations among its ontogenesis and its mature behavior connected with the problem of self/non-self discrimination.
Assuming a random repertoire for the self-antigen, we showed that, at first, the system is able to learn these antigens and to avoid attacking them even at successive equilibrium, then, we showed that this learning mechanism, on the experienced self, increases the average weighted connectivity of the resulting network of interacting lymphocytes as well as its variance: this is an important bridge among ontogenesis and mature repertoire as, from initial clonal deletion, the system can manage more reactive antibodies against the pathogens (low connected clones) and more anergic self-directed ones (of course within the Varela and Coutinho viewpoint), confirming both the possibility and the utility of the two mechanisms. Future development should include T-helper interactions (which may spread the phenomenon on several time-scales due to the intrinsic three-body interactions on diluted network [9]) as well as a systematic exploration of the relation among the amount of stored self-antigens in ontogenesis with respect to stability of the mature response against the number of encountered pathogens, so to understand the stability region of this system, where it works as a pattern reconstructor, with respect to its breakdown (so to try a statistical mechanics approach to memory saturation in the immune networks).

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