Some thoughts on the ontogenesis in B-cell immune networks

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Abstract In this paper we focus on the antigen-independent maturation of B-cells and, via statistical mechanics tools, we study the emergence of self/non-self discrimination by mature B lymphocytes. We consider only B lymphocytes: despite this is an oversimplification, it may help to highlight the role of B-B interactions otherwise shadowed by other mechanisms due to helper T-cell signalling. Within a framework for B-cell interactions recently introduced, we impose that, during ontogenesis, those lymphocytes, which strongly react with a previously stored set of antigens assumed as "self", are killed. Hence, via numerical simulations we find that the resulting system of mature lymphocytes, i.e. those which have survived, shows anergy with respect to self-antigens, even in its mature life, that it to say, the learning process at ontogenesis develops a stable memory in the network. Moreover, when self-antigen are not assumed as purely random objects, which is a too strong simplification, but rather they are extracted from a biased probability distribution, mature lymphocytes displaying a higher weighted connectivity are also more affine with the set of self-antigens, ultimately conferring strong numerical evidence to the first postulate of autopoietic theories (e.g. Varela and Counthino approaches), according to which the most connected nodes in the idiotypic network are those self-directed.

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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 number of experimental works increases, the need for minimal models able to offer a general framework where to properly locate experimental findings is a must for modelers interested in this field.

The purpose of the immune system is to detect and neutralize molecules, or cells (generically called antigens) potentially dangerous for the body, without damaging healthy cells [1]. The humoral response performed by B lymphocytes consists in analyzing the antigen, then the clone/s with the best matching antibody undergoes clonal expansion and releases specific immunoglobulins, which, in turn, are able to bind and neutralize pathogens. In order to achieve this goal, the immune system needs an enormous number of different clones, each having a particular receptor for antigens. As these receptors are generated at the genetic level randomly via somatic mutation, the body may produce lymphocytes attacking not only dangerous invaders (e.g. viruses), but also internal agents. The latter are referred to as self-reactive lymphocytes, which, if not carefully checked, may induce autoimmunity, an obviously unwanted feature.

In order to avoid auto-immunity, at least two mechanisms are thought to work: B-cells are generated, and maturate, in the bone marrow, where they are exposed to the so-called "negative selection rule"[1]. More precisely, 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) are induced to apoptosis, in such a way that only B-cells unable to attach to the available self survive[2] and share the freedom of exploring the body thereafter [1].

In fact, it is widely accepted that the bone marrow produces daily $\sim 10^7$ B cells, but only $\sim 10^6$ are allowed to circulate in the body, the remaining 90% undergo apoptosis since targeted as self-reactive [24]: as shown for instance by Nemazee and Burki [16], this depletion of the potential defense is due to the negative selection (clonal deletion) of immature B-cells expressing self-reactive antibodies or too low reactive ones.

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. Hence, another mechanism must act at peripherals levels (i.e. in the lymphonodes, spleen and liver). Indeed, Goodnow was able to show experimentally [7] that these self-reactive lympho-

[1] We only stress here that there exist strong differences between B-cell maturation in the bone marrow and T-cell maturation in the thymus [6, 14, 15]. Unlike TCR (T cell receptor), that evolved to recognize characteristic patterns of pathogens, BCR (B cell receptor) 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 [11].

[2] Strictly speaking, negative selection requires that newborn lymphocytes also display a non-null binding strength with at least a self-antigen, probably to avoid antibodies completely cut off from the host [10].

cytes actually exist in the body but, instead of undergoing apoptosis, they experience anergy in their responses, namely, under a proper stimulus, they do not respond. The main strand for explaining anergy and the consequent ability for self/non-self discrimination is via helper double signalling [11], nonetheless other mechanisms are expected to cooperate. Among these, collective features due to interactions among mature B cells may play a role and shall be investigated here trough statistical mechanics simulations.

The plan of the paper is as follows: in Sec. 2 we describe how the idiotypic network is generated and its main features; in Sec. 3 we develop the first approach to ontogenesis modeling, where we arbitrarily label as "self" a given amount of randomly generated antigens and check the subsequent growth of the network made of lymphocytes unable to attack these self-antigens. In Sec. 4 we develop an alternative approach, where we remove the (biological unreasonable) hypothesis that self-proteins are random objects and we deal with "correlated" self-antigens; impressively we find that in the correlated case the final repertoire not only correctly avoids to attach self, but also displays the peculiar topological structure suggested by Varela and coworkers, namely that nodes with high weighted connectivity are all self-directed. Finally, Sec. 5 contains discussions and comments on our results.

2 The minimal model

In this work we rely on the model introduced and developed in [2, 3], which achieves a description of the B-cell network able to recover as "emergent properties" basic facts such as low-dose tolerance, bell-shape response, memory features and self/non-self discrimination. However, within that framework the ability of the system to discriminate between self and non-self was recovered only at a cooperative level, in agreement with Varela and Coutinho [12, 20, 23]: clones which poorly interact with others are thought of as non-self-directed since they can easy respond to external fields (roughly speaking are more approximable as single particles), while clones which interact strongly and with a large number of other clones are thought of as self-directed since they experience a deep quiescent signal from nearest neighbors, which keeps them in a state of anergy. Here we want to move over and show that such mechanism regulation stems from and works synergically with negative selection.

Before proceeding, we briefly summarize the main features characterizing the interactions between B lymphocytes, ultimately leading to an idiotypic network; for more details we refer to [2, 3].

The system is made up of an ensemble of N different clones, each composed of M identical lymphocytes; a given lymphocyte 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). The antibodies secreted by a lymphocyte carry the very same idiotipicity expressed by the receptors of the secreting B cell. A

generic antibody is represented by a binary string of length L, encoding the expression of L epitopes[3]. In order to check immune responses we need to introduce the N order parameters m_i as local magnetizations:

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

From the magnetizations $m_i \in [-1,1]$, we can define the concentrations of the firing lymphocytes belonging to the i^{th} family as $c_i(t) \equiv \exp[\tau(m_i(t)+1)/2]$, where $\tau = \log M$, (see e.g. [22, 25]). Further, we introduce the Hamiltonian H which encodes the interactions among lymphocytes as well as the interactions between lymphocytes and the external antigens:

$$H = H_1 + H_2 = -N^{-1} \sum_{i<j}^{N,N} J_{ij} m_i m_j - c \sum_{i}^{N} h_i^k m_i, \tag{2}$$

where J_{ij} represents the coupling between clones i and j, while h_i^k represent the coupling between the clone i and a given antigen k (still represented by means of a binary string of length L) presented to the system and whose concentration is tuned by c.

The interaction matrix $\mathbf{J}$ and, similarly, the couplings $\mathbf{h}$, are built up as follows [2, 3]. Given two strings ξ_i and ξ_j, representing the idiotipicity of two clones, their μ-th entries are said to be complementary, iff $\xi_i^{\mu} \neq \xi_j^{\mu}$ so that the overall number of complementary entries $c_{ij} \in [0,L]$ can be written as $c_{ij} = \sum_{\mu=1}^{L} [\xi_i^{\mu}(1-\xi_j^{\mu}) + \xi_j^{\mu}(1-\xi_i^{\mu})]$. Following biological arguments the affinity between two antibodies is expected to depend on how much complementary their structures are, hence, we introduce the functional

$$f_{\alpha,L}(\xi_i,\xi_j) \equiv [\alpha c_{ij} - (L - c_{ij})], \tag{3}$$

where $\alpha \in R^+$ quantifies the difference in the intensities of attractive and repulsive contributes. Notice that $f_{\alpha,L}(\xi_i,\xi_j) \in [-L, \alpha L]$ provides a measure of how "affine" ξ_i and ξ_j are. When the repulsive contribute prevails, 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.

As mentioned above, the generic antigen presented to the system can be modeled as well by means of a binary string ξ_k and the rules determining the interaction strength between the i-th clone and the antigen are the same as for interaction between two antibodies, hence leading to the coupling h_i^k.

We finally recall that, from a statistical mechanics perspective, the Hamiltonian, calculated for a given configuration of magnetizations $\{m_i\}_{i=1,\dots,N}$, represents the

[3] The string length is assumed to be the same for any antibody following the fact that the molecular weight for any immunoglobulin is accurately close to $15 \cdot 10^4$ [9]

"energy" pertaining to that configuration and, according to thermodynamic prescriptions, the system spontaneously tries to rearrange in order to minimize it. Since the coupling matrix is symmetric, it is possible to construct a dynamics satisfying the detailed balance and relaxing to Maxwell-Boltzmann distribution [22]. In the following this is realized using a standard Glauber single spin-flip dynamics.

We also stress that, as simulations with a realistic amount of clones are prohibitive in terms of CPU time, we worked at smaller repertoire sizes and tested the robustness of results trough finite-size-scaling analysis (see Fig.1).

3 Random Ontogenesis

As we mentioned, during ontogenesis, those B-cells interacting strongly with self-antigens undergo negative selection and are deleted. Here we mimic this process by implementing the following learning rule: At the beginning, and once for all, N_S vectors are randomly drawn from a uniform distribution and stored as "self". Then, we extract sequentially and randomly (again from a uniform distribution) new strings representing newborn lymphocytes and those which are able to bind strongly to at least one self-antigen from the set N_S are killed, otherwise they are retained to build up the mature repertoire. The process is iterated until the size N for the repertoire is reached.

The resulting system is therefore characterized by the parameters N, N_S, L, α, where the interaction ratio α is kept fixed and equal to $\alpha = 0.7$ following biological evidences [2] and we also fix the scaling between N and L as $L = \gamma \log N$, according to bio-physical arguments [2]; here we choose $\gamma = 3$. As for N_S, we take it equal

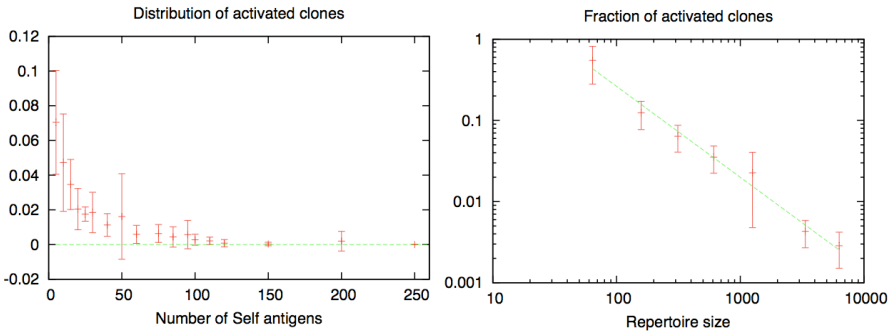

Fig. 1 Left: Distribution of the activated clones for an immune network at rest built up by $N = 628$ clones versus the amount of self-antigens used to generate the repertoire with antibodies made of by strings of $L = 11$ epitopes. Right: Finite size scaling of the system. Averaged response of the network 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 fit with N^x gives $x \sim -1.12$).

to a fraction of N. This allows to fulfill the relatively small survival probability for newborn B cells [24] and still retains a set of self-antigens vanishing with respect to the whole set of possible antigens, i.e. $N_S/2^L < \exp(-L(\log 2 - 1/\gamma))$. Of course, when $N_S = 0$ the original model [2, 3] is recovered. Finally, the binding between a newborn B and a self-antigen is considered to be strong if the number of complementarities between the related strings is larger than $3L/4$.

3.1 Results: Self-tolerance, memory and saturation.

Once the repertoire has been created, external antigens are presented to it and responses are checked. First, we test its ability in self/non-self discrimination by presenting to the system a field composed only by self-antigens and measuring the resulting magnetization. Indeed, we find that anergy to self is completely fulfilled (not shown in plots), for each experienced field made of by $1,..,N_S$ self-antigens. Conversely, when antigens presented do not belong to self, the fraction of the activated clones grows as the number of antigens presented increases, as reported in Fig.2 (left), eventually falling into a chronic activation state. This behavior can be easily understood from the perspective of spin glasses, due to the analogy between the system under study and a diluted random field model in the presence of a magnetic field: at low temperature, it undergoes a first order phase transition for a critical value of the external field [13, 5].

Furthermore, by enlarging the set of self-antigens N_S (at fixed repertoire size N), the matching between a generic self-antigen and the mature repertoire gets sharper so that only a small amount of highly affine clones is able to respond (see Fig.1, right).

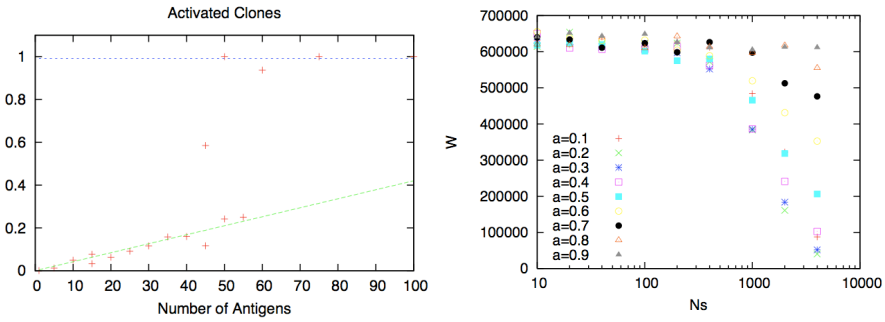

Fig. 2 Left: Fraction of the activated clones as a function of the antigens presented to the system: The system is made up of by 3352 clones and 50 self-antigens. Right: Averaged weighted connectivity for different repertoires generated increasing the size of the experienced self N_S at ontogenesis. For the latter various degree of correlation a have been tested as explained by the legend. Here we fixed $N = 2N_s$ and $\gamma = 3$.

4 Correlated Ontogenesis

Despite a certain degree of stochasticity seems to be present even in biological systems [1], self-proteins are not completely random objects [26, 18]. In order to account for this feature we now generate the repertoire of self-antigens according to a probability distribution able to induce a correlation between epitopes of self-antigens. Seeking for simplicity we adopt the following

$$P_{self}(\xi_i^\mu = +1) = (1+a)/2,\ P_{self}(\xi_i^\mu = 0) = (1-a)/2,$$

where a is a parameter tuning the degree of correlation: when $a = 0$ we recover the unbiased situation described in the previous section, while increasing (decreasing) $a \to +1$ ($a \to -1$) we move towards stronger correlation.

Figure 3 shows that the correlation between the weighted degree of a node and its affinity with self is numerically confirmed, and turns out to be larger for intermediate values of a. As a result, the system is expected to respond more strongly to non-self antigens, consistently with an healthy behavior (see Fig.3, right and Fig.4, left).

5 Discussion

In this paper we investigated the effects of negative selection occurring during the ontogenesis of B-cells. First we showed the ability of the system to develop memory of the self experienced at ontogenesis, in such a way that cells self-directed behave anergetically even in the mature repertoire. We also get a numerical confirmation of Varela's suggestions [20, 23], according to which nodes with high (weighted) con-

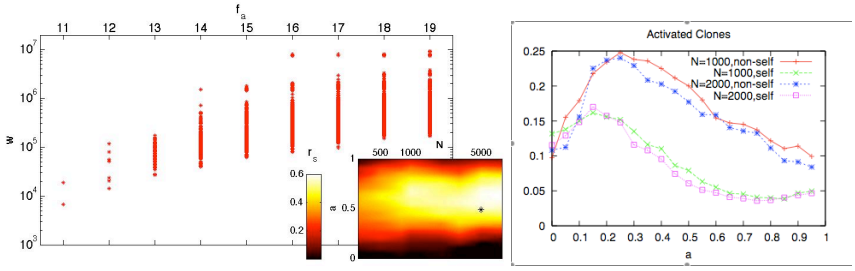

Fig. 3 Left: The figure envisages the correlation between the weighted degree w of a node and its (maximum) affinity f_a with the strings stored as self. Notice that larger values of w correspond to larger values of f_a. Such correlation has been measured in terms of Spearman correlation coefficient r_s which has been represented in the inset as a function of a and N. The black star $*$ corresponds to $N = 5000$ and $a = 0.45$, which are the parameters used for the main plot. Right: Fraction of activated clones as a function of a and for different sizes of the repertoire, as explained by the legend. The response of the system is measured in the case a self-antigen (dotted lines) and a external, non-self antigen (continuous line) is presented.

nectivity can be looked at as "self-directed". Therefore, ontogenesis acts as a learning process that, from one side, teaches to each single lymphocyte not to attack the proteins seen during maturation, and on the other side induces a correlation among idiotipicity yielding a possible regulatory role for the mature B-cell network. In this way, Varela's assumption is moved from a postulate to a physical consequence of a correlated learning process.

In this process a fundamental requisite is that self-proteins are not purely random object, but they share a certain degree of correlation. Here we introduce this bias in the simplest way just to show the idea; more biological patterns can possibly be implemented.

Future development should include T-helper interactions as well as an exploration of the relation between the amount of stored self-antigens in ontogenesis and the stability of the mature response against the number of encountered pathogens.

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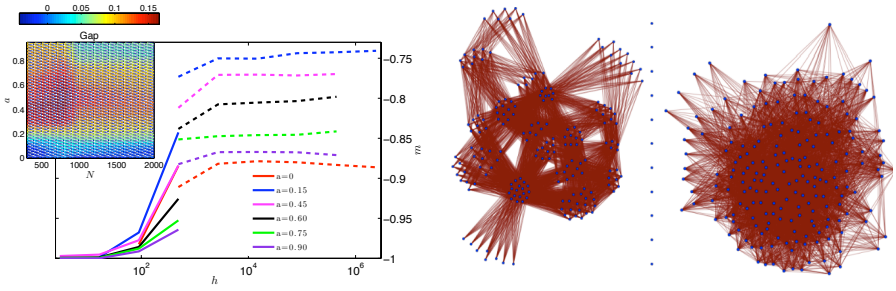

Fig. 4 Left: the main plot shows the local immune response, in terms of clonal magnetization m, versus the resulting field h due to the presentation of an antigen, either self (continuous line) and non-self (dotted line). The clone considered for the measure of the system response is the one with larger affinity with the string presented. Here we fixed $N = 5000$ and $\gamma = 3$. Moreover, several degrees of correlation a for the stored self are considered, as shown by the legend. For any of them there exists a value h^*, which typically works as upper bound for fields generated by self agents and as lower bound for fields generated by non-self agents. Interestingly, at h^* the resulting magnetizations exhibit a gap $m_{\text{non-self}}(h^*) - m_{\text{self}}(h^*)$, which is shown in the inset as a function of a and N. Right: Examples of the resulting idiotypic network with $a = 0.25$, $N = 200$ and $N_S = N$ (right) and $N_S = 2N$ (left).

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