Positive and negative selection, self-nonself discrimination and the roles of costimulation and anergy

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Supplementary Material

SM1- Details on numerical algorithm

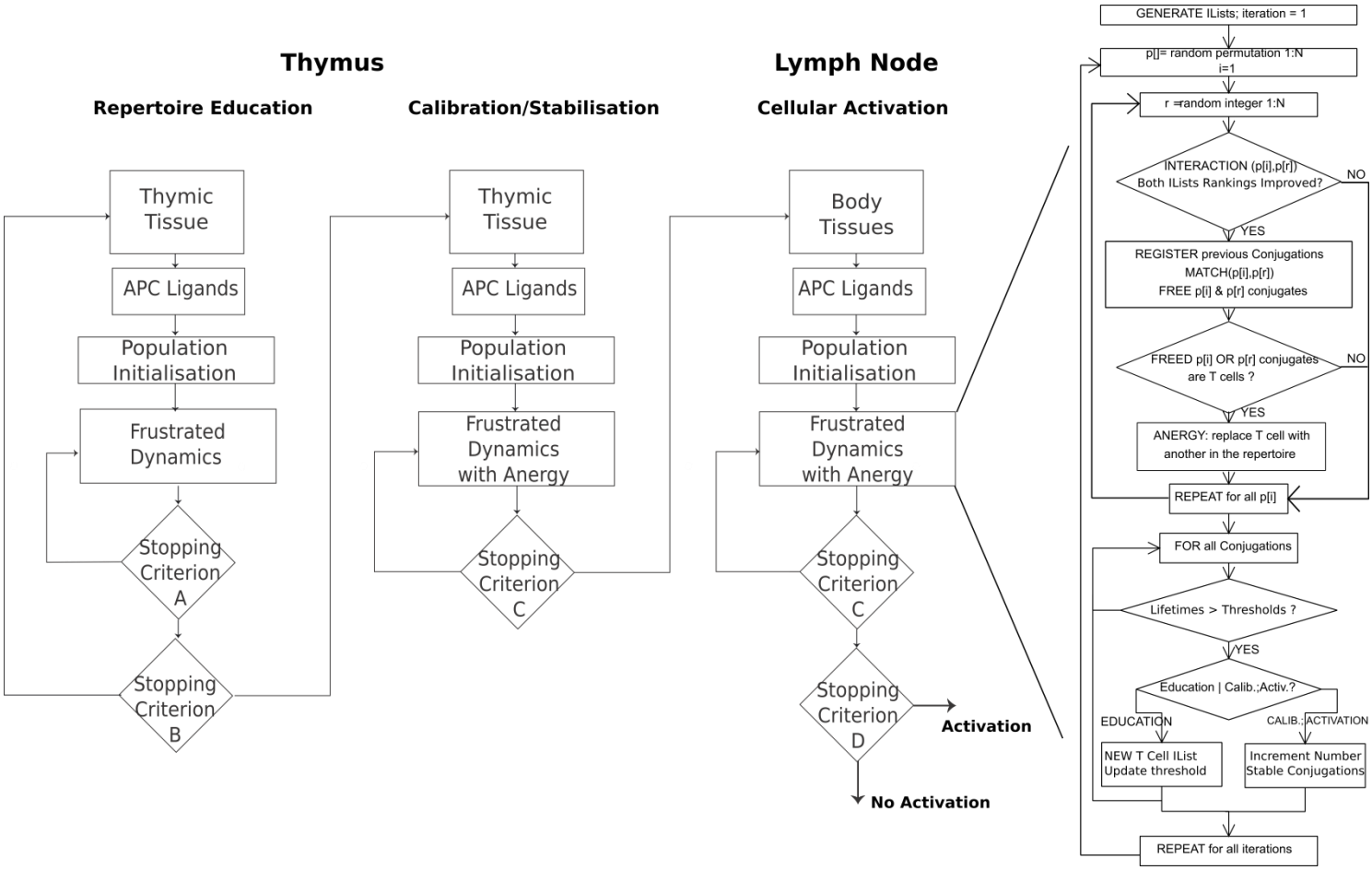

Figure SM1: Flowchart with the main steps involved in the numerical simulations used in this work. During repertoire education, negative selection is applied until a pre-defined Threshold (Stopping Criterion A); several T cell populations can be educated if necessary (Stopping Criterion B) to form a large T cell repertoire; after forming the whole repertoire, the frustration dynamics with anergy is run for W iterations (Stopping Criterion C) just as it will be used during cellular activation in lymph nodes. In this way characteristic lifetimes are extracted to calibrate the model; if an APC exceeds a number given of long contacts consistent with R>1, cellular activation is triggered (Stopping Criterion D). The Frustration Dynamics used during the education stage only differs from the Frustration Dynamics in the following stages, by the absence of the Anergy step.

SM2- Numerical experiments on the impact of anergy on the frustrated dynamics

Anergy continuously replaces the set of T cells engaging in the frustrated dynamics by other T cells in the repertoire. Given the large variety of ILists, many different T cells can establish maximally frustrated sets. For any population of T cells there will be some T cells engaging in less frustrated dynamics than the others. This results from the specific ordering of ILists. However, any T cell could be the less frustrated T cell in the population, as all T cells are equivalent on average. Hence, anergy should make conjugation lifetimes more uniform. This is indeed confirmed in Figure SM2. Note in particular that the impact of poorly educated T cell ILists is considerably reduced, making the maximum conjugation lifetimes smaller, and thus improving the ability to discriminate self from nonself.

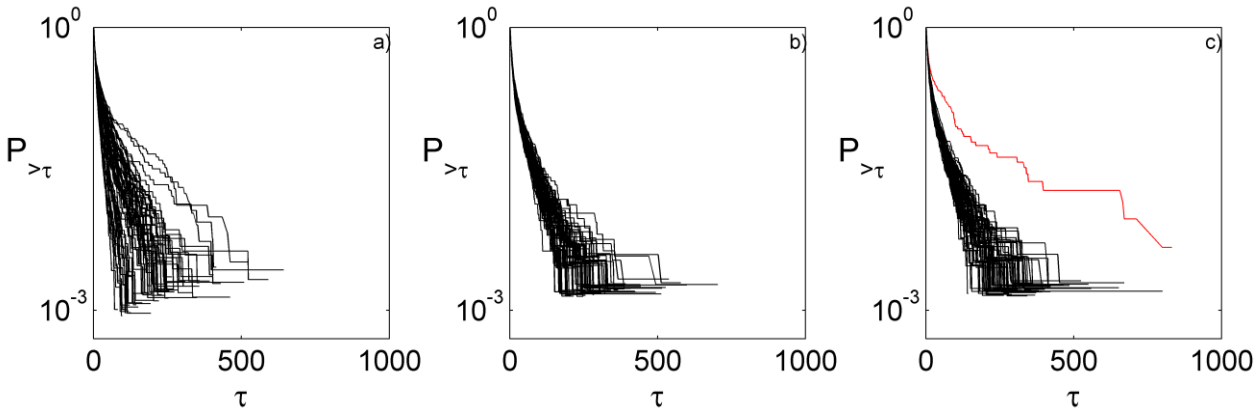

Figure SM2: Impact of the anergy on APCs conjugations lifetime. a) no anergy; b) anergy with a T cell repertoire made of 40 educated T cell populations; c) the same as in b) but with one APC displaying a nonself peptide. This should be compared with Figure 2, where no anergy had been applied. It is clear that discrimination becomes neater.

SM3 – A mathematical argument explaining the emergence of perfect self/nonself discrimination and the need for costimulation and anergy

We want to discuss why costimulation and anergy are necessary to achieve perfect self/nonself discrimination. During negative selection, T cell ILists having on top positions ligands displayed by APCs of the same subtype are eliminated, as they form stable conjugations with long lifetimes (see Figure SM3a). As a result ILists become ordered along the negative selection process, as it is shown in Figure 3 and schematically represented in Figure SM3b. The following discussion considers a population with 30 T cells per subtype, as in Figure 3, and that a foreign peptide is presented by a subtype I APC.

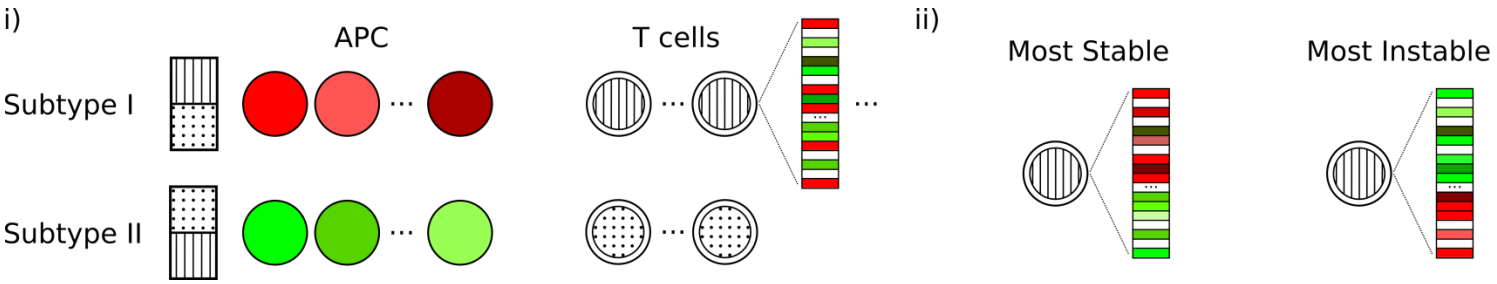

Figure SM3a: Representation of the set of cells in our model organized according to their cell type and subtype. Red and green colors represent respectively ligands presented by subtype I and subtype II APCs. Different intensities represent different ligands. APC ligands that have never been presented in the thymus are represented in white. T cells display only two ligands, represented by dots and vertical lines. ILists rank the other cell ligands according to decreasing order of affinity. All APCs belonging to the same subtype have the same IList. APCs can only have two possible ILists, since T cells display only two possible ligands. Since APCs display arbitrarily diverse peptides and T cell receptors are also very diverse, all T cells have different ILists which can rank APC ligands in very complex orderings as shown in i). In the following argument we concentrate of how subtype I T cells interact with subtype I APCs. T cells having on top positions red ligands (ligands displayed by subtype I APCs) establish stable conjugations with subtype I APCs. On the contrary, T cells having on top positions green ligands (ligands displayed by subtype II APCs) establish short lived (frustrated) conjugations (SM3a ii).

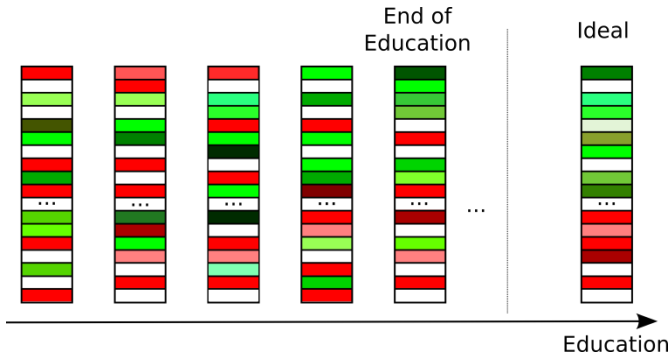

Figure SM3b: Illustration of the impact of the education process on the ordering of ligands in subtype I T cell ILists. In order to establish short lived conjugations, only subtype II ligands should appear on top positions of ILists. In an ideal education process, all top positions would be occupied by ligands represented in green. In practice, however, this is never achieved because the probability that such ILists are generated by random chance is negligible. Consequently, only top positions are ordered and even in the top positions some ligands represented in red can appear.

After negative selection, T cell ILists become ordered. If no self-ligand displayed by subtype I APCs appears in the top 10% positions of any subtype I T cell ILists then pathogen detection will be prompt and accurate. Indeed, contrary to self-peptides, foreign peptides have not shaped ILists during negative selection. Consequently, there is a 10% probability that subtype I T cells have on the top 10% positions the foreign peptide introduced in the system. Hence, the foreign peptide presenting APC will form distinctively more stable conjugations than conjugations formed by the other self-peptides presenting APCs. The response is prompt because these stable conjugations are established with 10% of the T cells from the same subtype, i.e., on average 3 T cells (Figure SM3c).

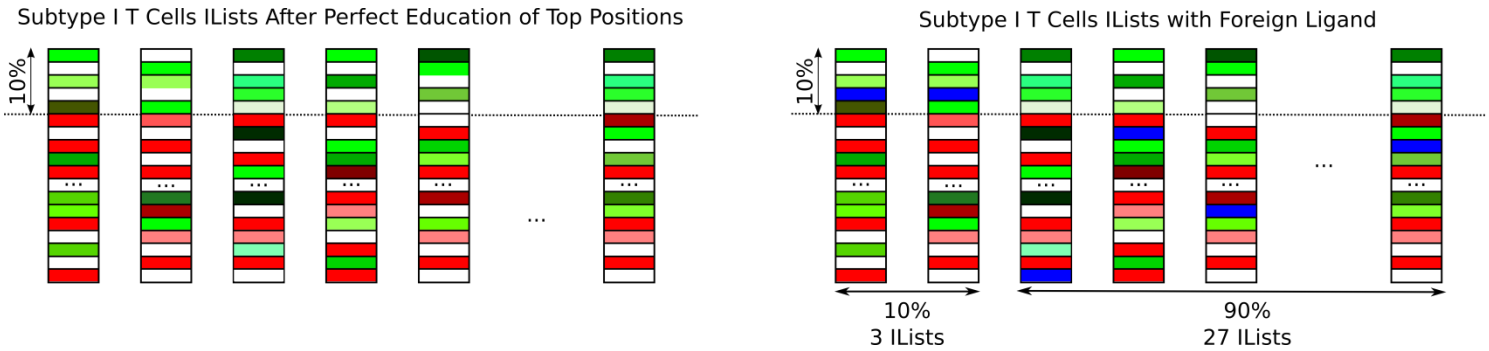

Figure SM3c: Illustration of the ordering of ILists after negative selection perfectly ordered the top 10% positions (left) and the effect of introducing a foreign ligand in the population. Here it is considered the extreme limit in which education did not place any ligand represented in red on the top 10% of ILists positions. Note that all ILists order ligands using very different orderings. When a foreign ligand is introduced in the population, it occupies any position previously represented in white. Since these ligands had never been in the system, they occupy random positions in ILists. Hence, 10% of ILists will have the foreign ligand in the top 10% positions. For a population with 30 subtype I T cells, this corresponds, on average to 3 ILists. However, due to stochastic fluctuations, in some cases there will be only 2 or 1 ILists with the foreign ligand in top positions.

The previous reasoning explains why self/nonself discrimination can be achieved. The mechanism requires that ILists can be ordered arbitrarily which highlights a driving force for the generation of T cell receptors diversity. However, a problem exists with the previous reasoning: it requires T cell ILists to be perfectly ordered, at least on the top 10% positions. But, from Figure 3 it is clear that this is not the case, as errors are made. Hence it seems more reasonable to assume that, with a 15% chance probability, subtype I self-peptides are placed on the top 10% positions of subtype I Tcells ILists. This agrees better with Figure 3.

As a result, on average, 30x0.15=4.5, i.e. 4 or 5 subtype I T cell ILists have subtype I self-peptides mistakenly placed on the top 10% of the positions; 4 or 5 different self-peptides can be mistakenly placed in top positions in different ILists. It can happen that the same self-peptide appears mistakenly placed in the top positions in 2 or 3 ILists. This is the number found for the foreign peptide. Hence, taking into account this stochastic effect, it seems hard to guarantee that the APC displaying the foreign peptide establishes a larger number of stable conjugations than all the APCs displaying self-peptides. Discrimination between self and nonself does not seem to be perfect.

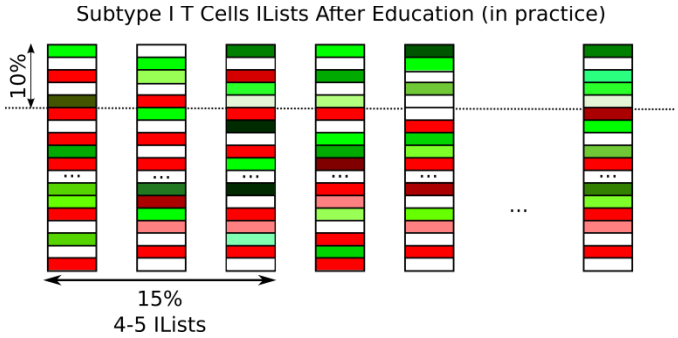

Figure SM3d: Illustration of the ordering of ILists after negative selection, in practice. There are 15% of ILists with a self-ligand on the top positions. For a population with 30 subtype I T cells, this corresponds, on average, to 4 or 5 ILists. The self-ligand in top positions could be the same in some ILists (2 or 3 ILists), just as it happens to the foreign ligand in some cases (Figure SM3b). Hence, it would not be possible to guarantee perfect self/nonself discrimination.

The importance of anergy will now become clear. Consider that every time a T cell is left non-conjugated it is sent to anergy, so that it is replaced by another T cell in the repertoire. Given that T cells are continuously replaced, the pool of T cells that interact with APCs is much larger. For a repertoire with 40 populations of educated T cell populations there are 40x30=1200 T cells of each subtype. The number of T cells that have the foreign peptide on the top 10% positions in their ILists is 40x3=120 T cells. The number of mistakenly placed self-peptides is 40x30x0.15=180. However, as all self-peptides have an equal probability of being mistakenly placed on top positions, then the average number of ILists with a given self-peptide in the top 10% positions is only 180/30=6 cells.

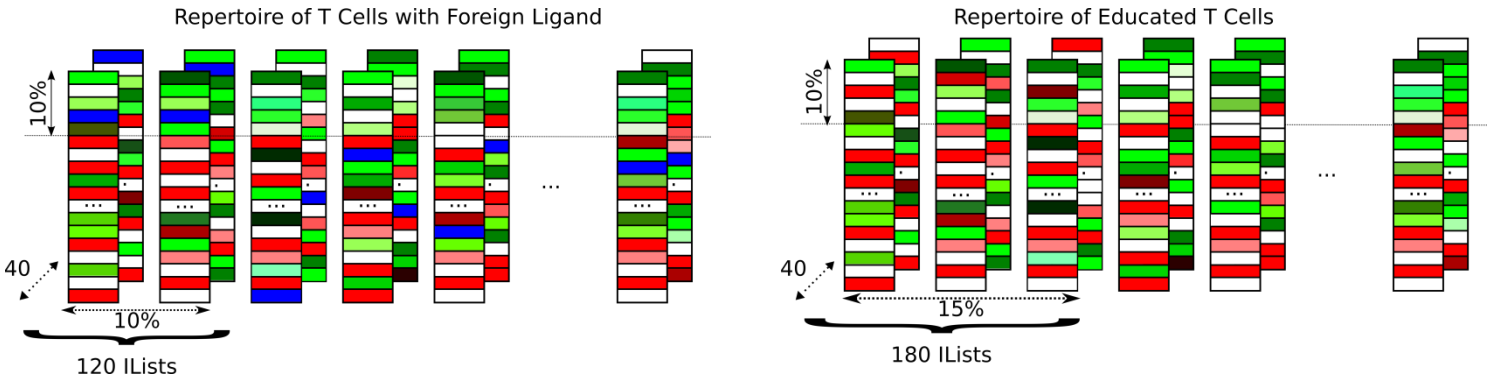

Figure SM3e: Illustration of the ordering of ILists for a repertoire made of 40 populations of T cells, with 30 subtype I T cells in each. There are 1200 subtype I T cells in total, and 10% of them (120 ILists in total) have the foreign ligand on the top 10% positions (Left). There are also 15% of the 1200 ILists that place self-ligands on the top positions, i.e., 180 ILists (Right). All self-ligands can appear with the same probability on these positions. Consequently, on average, each self-ligand appears in 180/30=6 top positions. Anergy allows APCs to interact with a large repertoire of T cells. Consequently, if activation is only triggered after a number of long contacts, APCs can have access to the information available on the repertoire, rather than on a subset of the total T cell repertoire.

To conclude, anergy makes discrimination between self and nonself accurate and free of the impact of stochastic fluctuations. For a repertoire of 40 T cell populations - 1200 subtype I T cells – the foreign presenting APC can establish stable conjugations with 120 T cells (10% in total), whereas APCs presenting self-peptides form stable conjugations with, on average, 6 T cells. Hence the rate of stable contacts is 20 times larger for APCs presenting foreign peptides. Costimulation should thus signal the detection of an abnormal rate of stable contacts.

This explains clearly why costimulation and anergy can work together to generate perfect self/nonself discrimination. The cellular frustration scenario also predicts that it is the APC that delivers the costimulation signal to T cells and not the inverse. Furthermore, it also explains why many T cells with very different specificities towards self-antigens can be activated by the same foreign antigen.

SM4- Impact of the extension of the repertoire education process on discrimination

Good discrimination between self and nonself does not necessarily require an exhaustive negative selection process. This can be concluded from the figure below, which shows that the quality of detection increases steeply with a quick negative selection process. Taken together, these results show that the self nonself discrimination mechanism is robust, not requiring extremely specific selection processes to achieve good results.

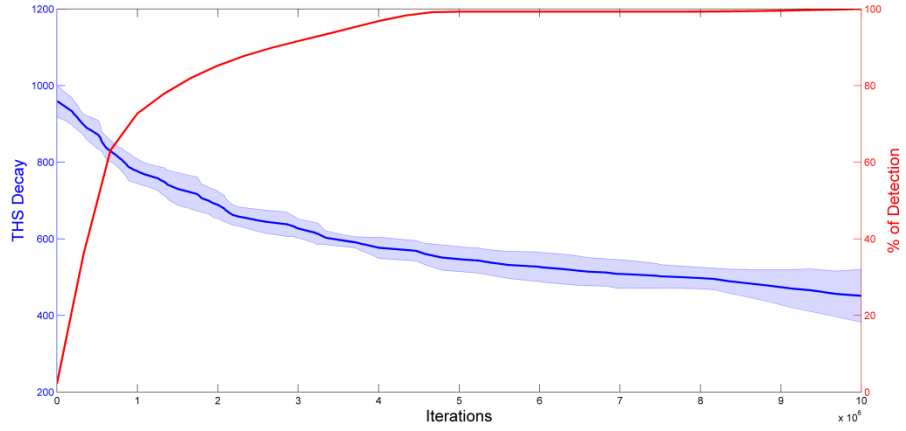

Figure SM4: Maximum conjugation lifetime (THS) as a function of the number of iterations used during the education process (blue curves) as opposed to the discrimination accuracy for populations educated for the number of iterations along the x axis.

SM5- Numerical Experiments on the impact of limited connectivity on repertoire education,

For large and diverse systems it is necessary to reduce each cell's connectivity to frustrate the dynamics. Below we show histograms of conjugations lifetimes for populations with 3 different connectivities per cell. In all cases education was taken for $2x10^7$ iterations. This number of iterations was chosen so that conjugation lifetimes cannot decrease much further. As it becomes clear from the analysis of the figure below, conjugation lifetimes are considerably reduced for smaller connectivities, corresponding to the onset of more frustrated dynamics.

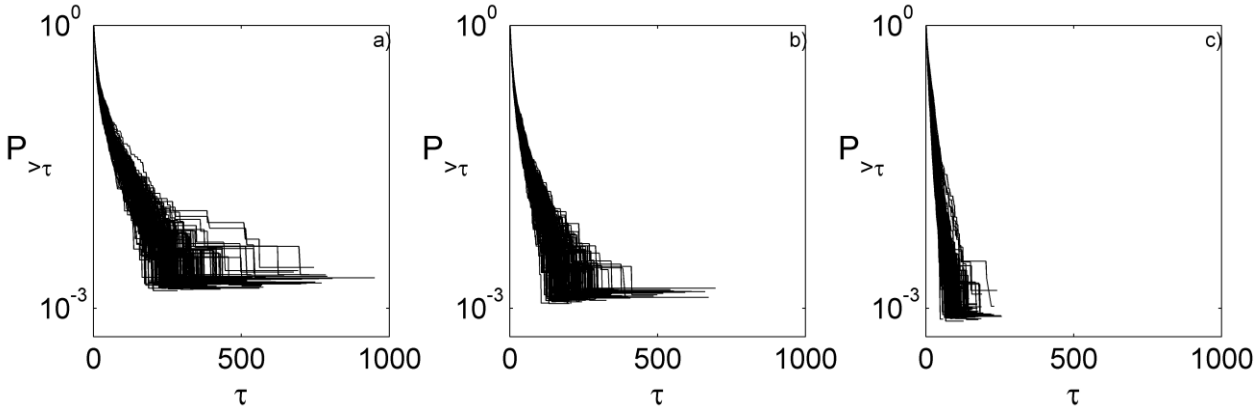

Figure SM5: Frequency of conjugations lasting longer than τ iteration steps for each APC in a population with 180 cells of each type. Each T cell could interact with a) 90 cells; b) 60 cells and c) 15 cells.

SM6- Numerical Experiments on the impact of limited connectivity on detection performances

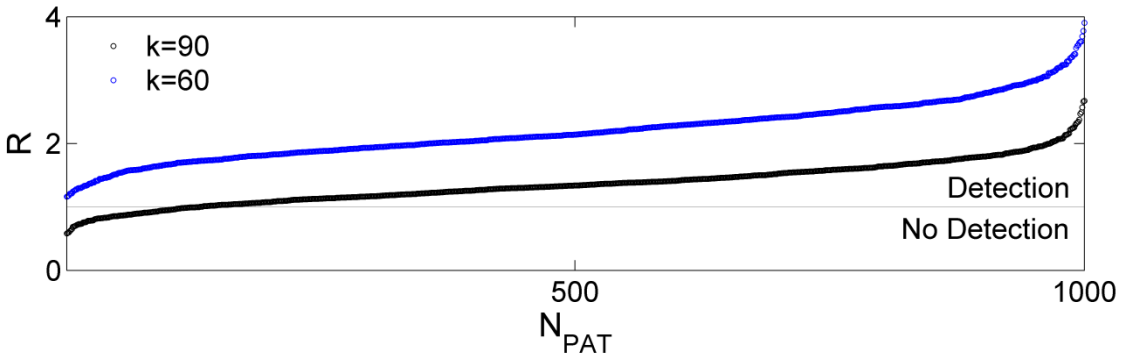

Figure SM6: Detection ratios calculated for a conjugation time equal to 15% of the maximum conjugation time registered during the education process in populations with 180 cells, i.e. $\tau_c = 112$ with connectivity K=90, and $\tau_c = 72$ with connectivity K=90. In both cases, T cell populations were educated for $2x10^7$ iterations. Perfect detection was achieved when K=60, agreeing with the results obtained for populations with 60 APCs. For K=90, 134/1000 foreign peptides were not detected (~13%), which agrees with the view that limited connectivity is important to achieve perfect nonself discrimination.

SM7- Derivation of the differential equations required to study the need for positive selection

Here we illustrate how one of the differential equations in (1) was derived. The other equations can be derived similarly.

Consider the equation for the evolution of the frequency of conjugations involving the APCs displaying ligand p_2 and $T_{I(21)}$ cells - subtype I T cells, with an IList with ligands p_2 and p_1, ranked in that order. The differential equation has two contributions. Positive contributions consider the rate at which $A_2T_{I(21)}$ conjugates are formed. This is proportional to the frequency of contacts that lead to the formation of an $A_2T_{I(21)}$ conjugate. Since subtype II APCs IList favor interactions with subtype II T cells, then these APCs can only form conjugates if they are non-conjugated. Note that if they were conjugated to a subtype I T cell they will not change pair since they do not receive a stronger signal from the $T_{I(21)}$ cell, also of subtype I. Concerning the $T_{I(21)}$ required to form the conjugate, it could be previously non conjugated, or conjugated to APCs displaying ligand p_1. Consequently, a part from a scaling factor that can be incorporated in a time scale, this contribution gives: $n_{2,\emptyset}\,(n_{\emptyset,I(21)} + n_{1,I(21)})$.

Concerning contributions destabilizing $A_2T_{I(21)}$ conjugates, all interactions with both cells in the conjugate should be considered. However, the T cell is conjugated to a cell that is ranked on the top of its IList. Consequently, the T cell cannot be destabilized. On the other side, the APC can be destabilized by any subtype II T cell. However, the subtype II T cell must also favor an interaction with an APC displaying ligand p_2. This can be achieved by i) any non-conjugated T_{II} cell that has ligand p_2 in its IList ii) any conjugated T_{II} cells that have ligand p_2 on the top of its IList. Consequently we get the contribution:

$$-n_{2,I(21)}(n_{\emptyset,II(12)} + n_{\emptyset,II(21)} + n_{\emptyset,II(23)} + n_{\emptyset,II(32)} + n_{1,II(21)} + n_{3,II(23)}).$$

The final equation then becomes:

$$\frac{dn_{2,I(21)}}{dt} = n_{2,\emptyset}\,(n_{\emptyset,I(21)} + n_{1,I(21)}) - n_{2,I(21)}(n_{\emptyset,II(12)} + n_{\emptyset,II(21)} + n_{\emptyset,II(23)} + n_{\emptyset,II(32)} + n_{1,II(21)} + n_{3,II(23)}).$$

From this equation we also get an expression for the conjugate lifetime:

$$\tau_{2,I(21)} = 1/(\tilde{n}_{\emptyset,II(12)} + \tilde{n}_{\emptyset,II(21)} + \tilde{n}_{\emptyset,II(23)} + \tilde{n}_{\emptyset,II(32)} + \tilde{n}_{1,II(21)} + \tilde{n}_{3,II(23)})$$

After negative selection, T cells establishing stable conjugations are eliminated, and this expression is simplified to:

$$\tau_{2,I(21)} = 1/\tilde{n}_{\emptyset,II(12)}$$

Similar expressions can be obtained for the typical time a cell remains non-conjugated.

SM8: Numerical results demonstrating the importance of positive selection on repertoire education

Consider a model in which APCs present 26 different peptides as shown in the figure below. There are APCs presenting 26 different ligands. Five APCs present ligands 21, 22, 23 and 24; ten APCs present ligands 25 and 26. Ligands between 1 and 20 are presented by one ligand each. Results in Figure SM8b demonstrate that for heterogeneous presentations as in the model in Figure SM8a, positive selection is crucial for reducing conjugation lifetimes when connectivities are small; for large connectivities positive selection can be redundant.

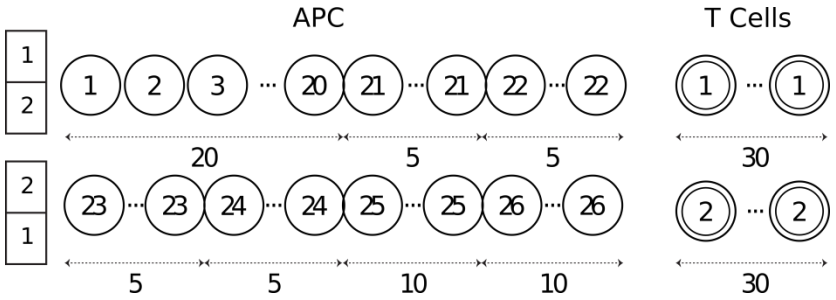

Figure SM8a: Model used to demonstrate the importance of positive selection in repertoire education

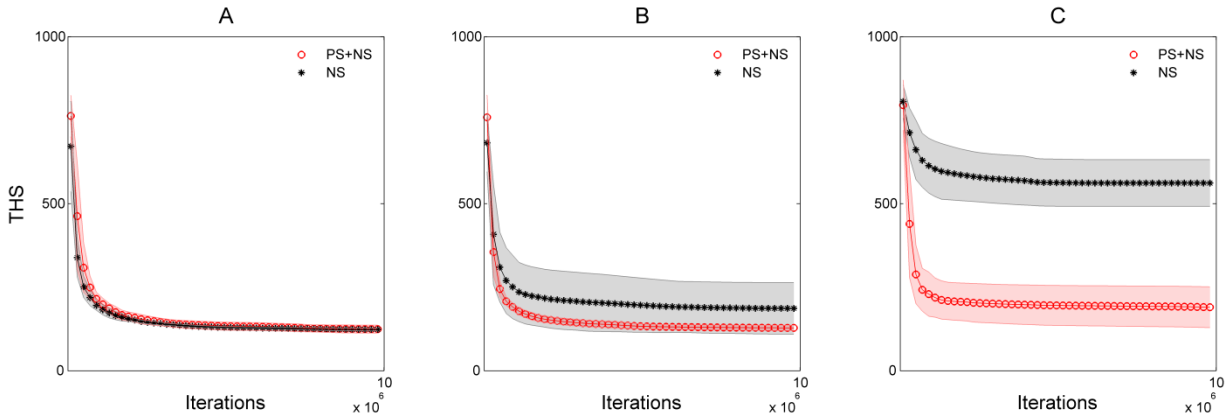

Figure SM8b: Results obtained for the model in Figure SM8a, for 3 different connectivities, with negative selection (NS) or negative and positive selection (PS+NS) for A) total connectivity, i.e., K=60 B) K=20 and C) K=10. Shaded regions represent standard deviations obtained with 10 populations.

SM9: Considerations on some model's assumptions and implications to the concept of self/nonself discrimination

The cellular frustration framework describes the dynamics of interactions between APCs and T cells, which depends on the information displayed by APCs and sensed by T cell receptors. How this information relates to the materials collected by APCs and on how APCs process and present this information to T cells is not specifically addressed in the model. Similarly, how the information displayed by APCs is ranked in ILists is also not specifically addressed. It is possible to consider that ILists rank the single peptide-MHC complexes displayed by APCs depending on the affinity of the interaction with T cell receptors – this is the view we privileged in the discussion. It is however also possible to assume that the listed ligand information is more complex and results from multiple interactions with a profile of peptides displayed by an APC.

Another point that is also not addressed specifically relates to the degeneracy of the interactions, and consequently to the number of ligands or different presentation profiles that are sensed by T cells as being equivalent. In the presence of degeneracy, foreign and self antigens can become indistinguishable. If all T cells cannot distinguish a foreign antigen from a self antigen, then they would not be detectable using the arguments discussed in this work. In that case, the detection of the proliferation of these antigens would require frequency dependent responses. This is in fact another property featured by cellular frustrated models and which will be addressed in a future publication.

SM10: Perfect self/nonself discrimination for populations in which APCs' presentation varies

The assignment of peptides to APCs can also be generalized for systems in which APCs present different configurations. In the example represented in figure SM10a, the set of self ligands is composed of 80 arbitrarily chosen numbers, representing peptides. Half of these peptides are presented by subtype I APCs, and the remaining by subtype II APCs. During positive and negative selection and the calibration phases (see SM1), every W=5000 iterations new subsets of peptides are randomly drawn and presented by the 30 APCs of each subtype in the system. An ordering of each subset is done before assigning peptides to cells, so that smaller numbers are presented by APCs with the smaller indices: this reduces the variability in the presentation by each APC. The number of possible configurations of peptides presented is $(C_{30}^{40})^2 \sim 10^{17}$. The number of configurations is thus so large that it requires that T cells gain the ability to generalize the set of peptides that can be presented and tolerated.

During the detection stage, a randomly drawn APC presents a peptide not belonging to the self-set and detection ratios were calculated as before. As can be seen in Figure SM10b, all 1000 foreign peptides presented were detected, which shows that our arguments also apply for systems in which presented configurations vary.

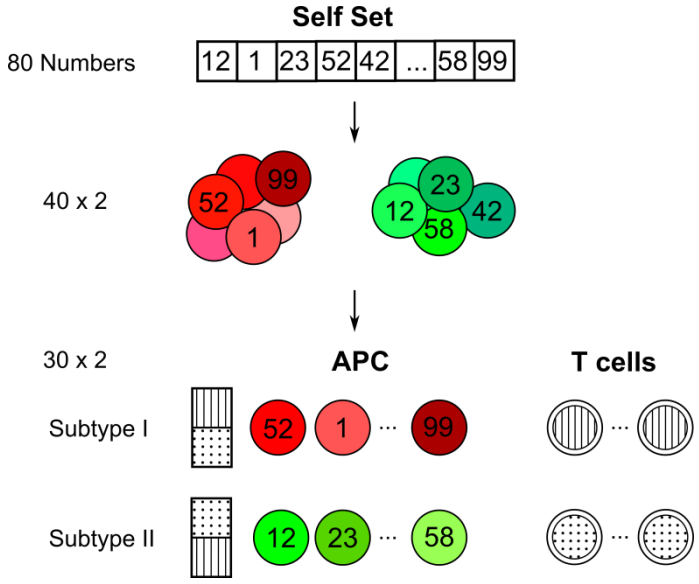

Figure SM10a: Description of the procedure used to generate a diverse range of peptide configurations presented in a model with 60 APCs. From an initial self-set with 80 arbitrary numbers, two sub-sets were defined from which samples with 30 numbers were drawn and presented by APCs of each subtype. APCs presentations were changed every W=5000 iterations. For the simulation of the dynamics in a lymph node, APCs display a single randomly sampled configuration of self-peptides and one APC presents a number that was not in the self-set.

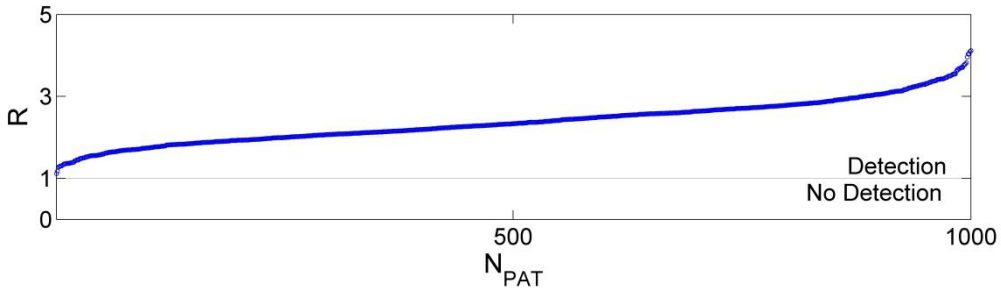

Figure SM10b: Detection ratios obtained for the model described in Figure SM10a, for 1000 different presented foreign peptides. The dynamics was run for 10000 iterations and the tolerance factor f was set to 1.05.