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Computer simulations of heterologous immunity: Highlights of an interdisciplinary cooperation

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Computer simulations of heterologous immunity: Highlights of an interdisciplinary cooperation

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Abstract

The relationship between biological research and mathematical modeling is complex, critical, and vital. In this review, we summarize the results of the collaboration between two laboratories, exploring the interaction between mathematical modeling and wet-lab immunology. During this collaboration several aspects of the immune defence against viral infections were investigated, focusing primarily on the subject of heterologous immunity. In this manuscript, we emphasize the topics where computational simulations were applied in conjunction with experiments, such as immune attrition, the growing and shrinking of cross-reactive T cell repertoires following repeated infections, the short and long-term effects of cross-reactive immunological memory, and the factors influencing the appearance of new clonal specificities. For each topic, we describe how the mathematical model used was adapted to answer specific biological questions, and we discuss the hypotheses that were generated by simulations. Finally, we propose rules for testing hypotheses that emerge from model experimentation in the wet lab, and vice-versa.

Keywords: IMMSIM simulator, heterologous memory, attrition, repertoire changes

Introduction

Antigen recognition and the phenomenon of cross-reaction

Nothing can be simple when two quite different and highly complex organisms meet and fight for survival. In the case of infectious diseases, the organism that is attacked is able to produce a specific immune response, while the “attacker” seeks to escape the hosts’ defence by continuously mutating. This simplified description implies that rarely the result of a viral infection is a clean victory of one of the two contenders: several “third endings” are possible, including chronic disease and chronic responses, with variations triggering autoimmunity, allergic responses, and all sorts of damages inflicted actively and passively to organs and tissues, in the form of immunopathology [1–3].

We assume that one of the reasons for the appearance during evolution of specific immunity in vertebrates was the advantage of concentrating resources and fast responses specifically against recognizable threats, without upsetting the equilibrium of the entire organism. This was possible with the appearance of “lymphoid” cells, first found in primitive fish, and of glycoproteins belonging to the immunoglobulin superfamily [2,4]. These glycoproteins carry a variety of receptors used to recognize pathogens and serve as building blocks for all immune receptors of effector and regulatory cells of both branches, humoral and cellular, of the immune system.

Through the mechanism of somatic recombination of germ line sequences, they can be engineered to produce billions of unique receptors to recognize antigens belonging to different pathogens [1,2,4].

However, even this large diversity can never be sufficient to cover a *one-to-one* recognition of the ever-mutating invaders. To face this limitation, suboptimal antigen-receptor bonds are allowed to reach the signal threshold, a phenomenon called cross-reaction (observed in both antibodies (Abs) and T cell receptors (TCRs) [2,5–7]).

Cross-reaction allows one receptor to bind, albeit with different affinities, to a variety of similar epitopes, and the same epitope to be recognized by a number of different receptors and clones of cells. Despite allowing for recognition of a great variety of antigens by using a fairly low amount of different genes encoding for the immune receptors, this compromise can be potentially dangerous for the host organism. For example, “imprecisions” can generate errors, such as mistaking self-antigens for foreign ones, and cause autoimmune interactions [8].

Immunological memory, cross-reactivity, and competition for space

Immunological memory has gained public interest by being named after a function of the mind. A loaded number of assumptions come along with the name, for example immutability and persistence. However, recent findings suggest that immunological memory is not immutable but extremely plastic during the lifetime of an individual.

The memory repertoire that forms after one infection or vaccination is composed of a certain number of clones, with each clone represented by a number of cells with a long, but not perpetual life. The relative number of cells per clone determines its speed and efficiency of intervention, and therefore its “rank” in the hierarchy of the (secondary) immune response [1,2].

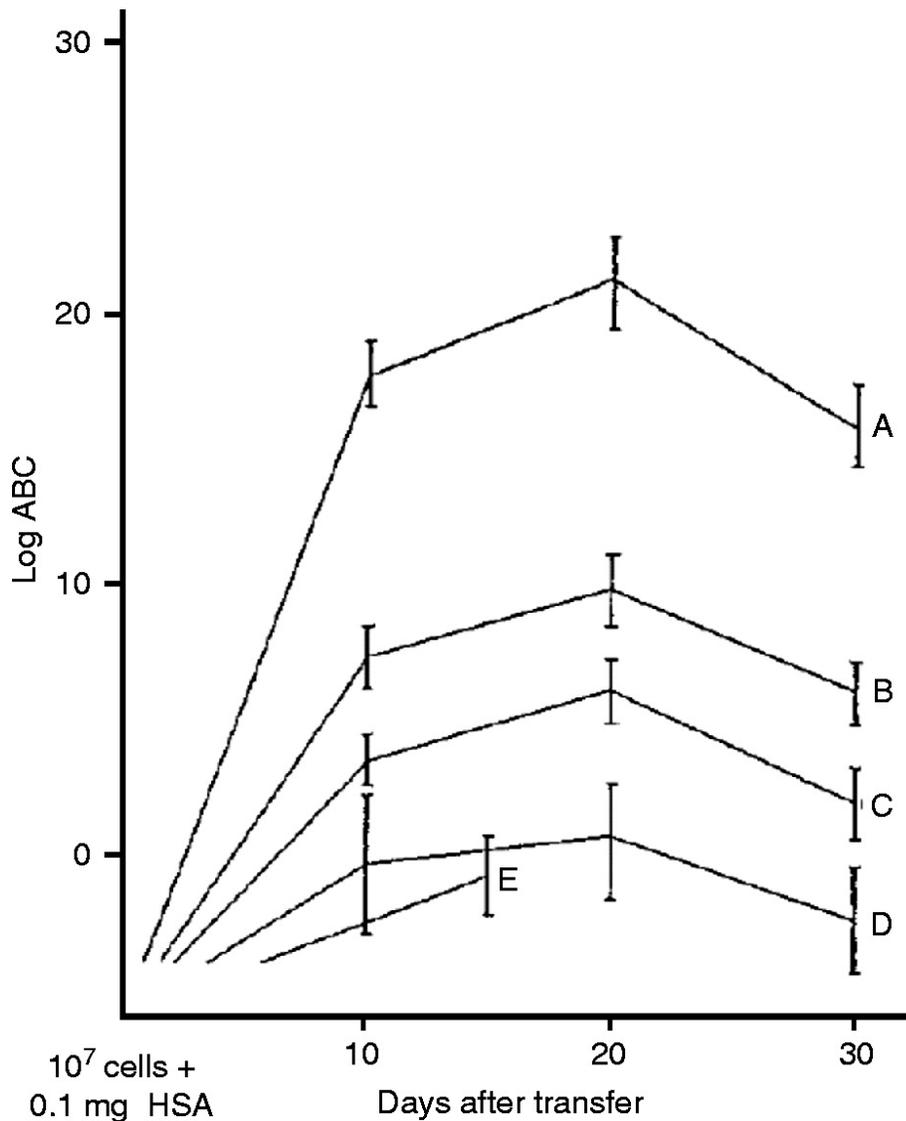
Cross-reactivity is one of the most important forces acting on the memory repertoire: the clonal repertoire of memory cells can change dramatically following any new infection with cross-reacting epitopes [3], with respect to both clonal composition and clonal hierarchy. The importance of clonal hierarchy and its changes following cross-reacting infections becomes even more evident if we consider that the immunological “space” for memory cells is certainly limited, either if interpreted as actual geometrical space for the cells to nest in, or in a more comprehensive way including vital resources and cytokine-loaded growth stimuli.

The importance of immunological space in affecting immunological memory was already explored and recognized by pioneering studies of “adoptive memory” in the mid-1960s. In these experiments, after priming of donor mice with human serum albumin (HSA), donor spleen cells were transferred into syngeneic recipients. Recipients were then challenged with a low dose soluble HSA and monitored for circulating Abs during the following month [9].

In these conditions, all antibodies (Abs) against HSA were generated by the transferred donor memory cells. Interestingly, antibody titers were low if the recipients were intact, but were up to 20 times higher if the recipients were previously irradiated (Figure 1). The hypothesis behind this finding is that irradiating the recipient created immunological space that allowed for the successful transfer of more donor cells. In addition, it was shown that the functionality of the transferred memory cells in non-irradiated recipients was influenced by the recipient's age, the memory response being better in very young mice and progressively worse in adults and old animals.

Figure 1. Antibody response originating from 10^7 donor spleen cells transferred: (A) into 500 R irradiated recipient mice; (B) 30 days old; (C) 39 days old; (D) 66 days old; and (E) 120 days old, non-

irradiated recipients. Groups of 6 to 10 mice. Plotted are mean log ABC \pm 95% confidence limits (reprinted with permission from [9]).



Despite not dissecting its very nature, this experiment suggests that immunological space is limited for memory cells. In addition, its dependence on age may indicate that space is influenced by a “growth factor”, thus being more of a functional than of a purely “geometric” entity [9,10].

Cross-reactivity and heterologous immunity

The phenomenon of cross-reaction in the context of T cell memory cells and its deep impact on immunological space has been extensively studied by the group of virologists led by Drs Welsh and Selin at UMASS, Worcester, MA, USA. Their studies have highlighted how cross-reaction is a fairly common event and how it can be regarded as one of the central cognitive principles used by the immune system during the response to viral infections. In particular, these studies focused on the phenomenon of

heterologous immunity, which is defined as the protection conferred by heterologous viruses against cross-reacting subsequent infections [5–7].

Despite conferring lower protection than homologous immunity (immunological memory against the same pathogen), heterologous immunity has been shown to have substantial impact on the outcome of viral infections. Heterologous immunity is also known to be fundamental in shaping the clonal diversity of the T cell memory repertoire, by causing cross-reactive clones to increase in number and non-cross-reacting clones to be reduced after subsequent infections, a phenomenon called “attrition” [11,12].

Attrition is defined as the long-term loss of memory cells upon infection, and is crucial in shaping the clonal hierarchy of memory cells after subsequent cross-reacting infections [13]. Changes in the dominance hierarchy of immunological memory were initially attributed only to competition for the limited geometric space available to memory cells. However, experimental results showed that, at the moment of stimulation, type-I interferon (IFN), a cytokine with lethal potential, was also secreted [12].

This finding suggests that the change in hierarchy may be more complex, and results from two types of attrition, a “passive” one (mere competition for geometrical space) with apoptosis of the less fit as an endpoint, and an “active” one, due to non-selective cytokine-induced early apoptosis of memory cells (“active” attrition). These phenomena are crucial in shaping the memory clones hierarchy, either by allowing high-affinity naïve clones to enter the memory repertoire (active attrition), or by favoring the proliferation of initially subdominant but cross-reactive clones, while reducing the numbers of initially dominant but non-cross reactive ones (passive attrition).

Despite the progress made in understanding the plasticity of the T cell memory repertoire following heterologous infections and the importance of immunological space, there are many aspects and factors relevant to the development and the outcome of heterologous responses that still have to be clarified. It is on these topics that the interdisciplinary collaboration presented here was launched.

In this review, we summarize and discuss the results of this collaboration, with particular attention to the contribution of modeling to the generation and verification of hypotheses for the wet lab. The topics addressed by simulations were mainly concerned with the cooperation and competition between the two branches of the immune system [9,14], the relationship between memory and naïve responses [15,16], and between cross-reacting clones with different binding affinities for the same epitope, which generates competition for clonal space [11,12], and consequently affects repertoires.

IMMSIM, a model of the immune system

Computer simulations were performed with IMMSIM, an agent-based model of the immune system governed by probabilistic events. The IMMSIM software is available at <http://www.immsim.org> and can be downloaded for research and educational use. Here, we give a short description of the model, mainly focused on the characteristics relevant to this review. A more detailed description of IMMSIM can be found in the original articles of our group [14,17–19] and in *Autoimmunity*, this issue [16].

The IMMSIM body consists of a grid of discrete “interaction sites” where a predefined number of cells of several types of the immune system (T helpers 1 and 2, T cytotoxic, B cells, and macrophages) and epithelial cells are distributed, meet with each other and with antigens, and mount an immune response whenever a virus infects target epithelial cells.

Immune specificity is represented in the model by means of binary strings, which encode both immune cell receptors and antigens. Binary strings are sequences of zeros and ones of a given length: complementarities between strings encoding receptors and antigens are defined by the presence of a “one” and a “zero” in corresponding positions. The number of complementary (matching) positions determines the affinity of a receptor for a certain antigen, while the length of the binary strings determines the diversity (number of different receptors and/or antigens) that can be achieved in a given simulation. The specific interactions between entities are governed by affinity, such that only immune cells carrying receptors with a certain number of matching sites are stimulated to produce a response. In addition, interactions are probabilistic, since they are governed by computer generated random numbers (RNs).

Different RNs result in responses different in repertoire and outcomes, simulating the variability within individual subjects *in vivo*: for this reason, we refer to simulations started with different RNs and different naïve cells repertoires as “virtual mice” (VM). Pathogens are defined by their antigenic specificity and by physiological characteristics, such as their ability to infect epithelial cells (infectivity), their speed of replication (speed) and their lethality (lethal load, defining how many units of pathogen cause an epithelial cell to die).

Adaptations and upgrading introduced to run the simulations illustrated in the present review are briefly noted here. Although the basic model was found to simulate heterologous responses, the length of binary strings representing receptors and epitopes was increased from 8 to 16, which guarantees a total diversity of 2^{16} (65536) for all specific determinants. Four degrees of affinity were allowed for successful receptor-antigen interactions (0, 1, 2, 3 mismatches allowing binding).

By assigning to each VM 2500 cells of each type carrying specific receptors, we obtained a credible albeit minimalistic diversity of repertoire for each specific antigen (up to 26 different clonotypes of B cells, T cytotoxic, and T helper cells). In the study of anti-virus cross-reactive T cytotoxic responses, viruses were always used in the minimal configuration of one “epitope” visible to the humoral branch and one covered “peptide”, presented on the major histocompatibility complex to the cellular branch, while their “physiological” characteristics (such as infectivity, speed of duplication, and lethal load) were set to allow complete clearance by homologous immune response.

Depending on experimental design, cross-reacting viruses were taken out at random, or constructed in couples (used for priming and challenging infections) of selected bit distances. In the study of attrition [13], the two configurations (“active” and “passive”) were obtained by introducing a decay routine, acting stochastically on the single cells. The routine can be activated by two independent triggers: one (mimicking passive attrition) is set off whenever a given memory pool level is reached; the other (for active attrition) is activated upon IFN secretion by infected epithelial cells.

Applications and results

Growing, shrinking, and focusing of memory repertoires upon heterologous infections

Immunologically naive hosts respond to infections differently than experienced hosts, partly because of the presence and activation of cross-reactive memory CD8 T cells that can mount a response against

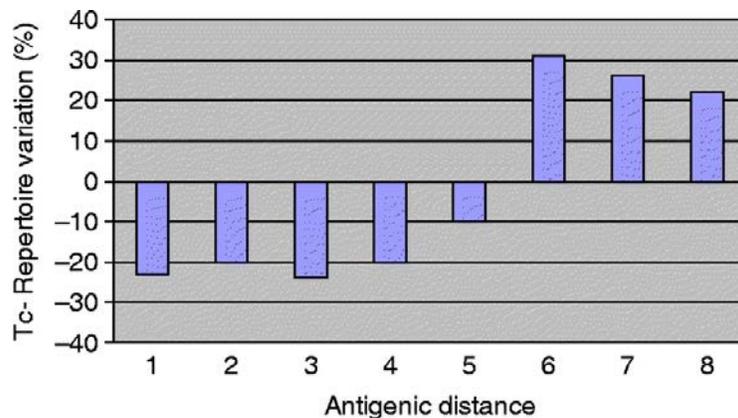
newly encountered pathogens. The presence of cross-reactive memory cells greatly impacts the size and the composition of the memory repertoire after heterologous infections.

Cross-reactive memory CD8 T cells can compete with the proliferation of naive CD8 T cells, even those that would normally be immunodominant during a primary immune response. This can lead to a change in epitope-specific T cell hierarchies upon expansion of a cross-reactive T cell population, which modulates clonal dominance and also results in a narrowed, more focused, oligoclonal TCR repertoire. However, experimental data show that the hierarchy of immunodominance within the narrowed repertoire is unpredictable and varies with the private, i.e. unique, specificity of the host's TCR repertoire [20].

During our collaboration, we investigated with simulations the changes in both size and composition of the T CD8 memory repertoire upon both homologous and heterologous challenges.

Upon cross-reacting challenge, it is reasonable to expect that the fraction of re-called memory clones would decrease with increasing antigenic distance, while the fraction of stimulated naïve cells should increase. Our simulations confirm these assumptions and reveal a “biphasic” behavior [15]. Our results show that homologous secondary responses and heterologous cross-reactive responses of near and medium antigenic distances shrink the size of the T memory repertoire. On the contrary, heterologous responses against pathogens antigenically distant or unrelated significantly increase the size of the repertoire (Figure 2).

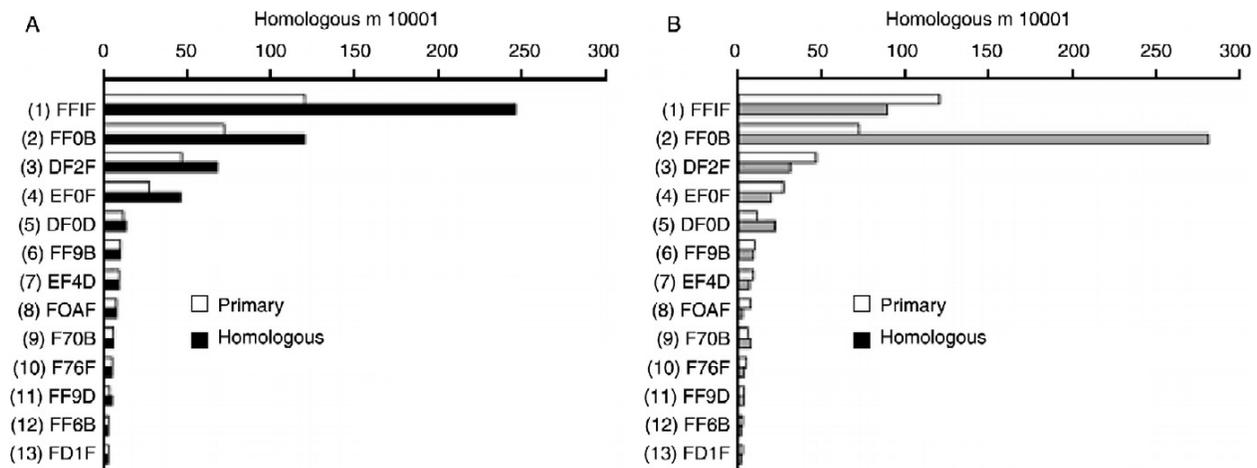
Figure 2. Ordinate: Percent variation (+ or -) of TC memory repertoire after secondary response. Ordinate: Percent variation (+ or -) relative to primary clonality. Abscissa: Antigenic distance between priming and challenging antigen. At $D = 0$, the same antigen is used for priming and challenging. At $D = 1, 2, 3$, the number of cross-reacting memory clones is progressively decreasing. At $D = 5, 6$, some cross-reaction is still possible. At $D = 7$, no cross-reaction is possible and the two antigens are perceived as foreign to each other.



The focusing of the T cell memory repertoire upon homologous and heterologous challenges was also simulated by computer modeling. The “uniqueness” of each VM repertoire was simulated by using a sufficiently large theoretical diversity of TCRs (2^{16} different receptors) and by assigning to each VM a limited number of naïve cells, so as to minimize the overlap among repertoires in different mice. Figure 3 shows a representative simulation comparing homologous versus heterologous challenge, where

narrowing of the clonal distribution was monitored by calculating the increase in the skewness of the repertoire. Skewness is a measure of the degree of asymmetry of a distribution, and in this case it is used as a measure of the narrowing or focusing of the memory repertoire. The skewness of the memory repertoires after primary infections and after homologous and heterologous challenges was compared using a two-tailed unpaired *t*-test; *p* values < 0.05 were considered significant. Our results are in agreement with biological data and show that [11]: (a) homologous challenge only weakly focuses the repertoire (no statistically significant difference in the skewness before and after challenge, $p > 0.05$, Figure 3(A), left); (b) heterologous challenge results in altered clonal dominance and significant narrowing of the pre-existent memory distribution (Figure 3(A), right; $p < 0.01$). In addition, a significant difference was found between the average skewness of homologous versus heterologous challenge of the same memory population (evaluated across 30 simulations), with the index being higher in the heterologous challenges ($p < 0.01$).

Figure 3. Computer simulation of homologous versus heterologous virus challenge. (A) Left: Clonal distribution of the memory population before (white bars) and after (black bars) a homologous challenge. (B) Right: The same population before (white bars) and after (gray bars) a heterologous challenge. The x axis shows the absolute number of CD8 T cells, while y axis labels indicate hexadecimal representation of each clone involved in the response. The number in brackets on the left of the clone labels represents the place occupied by a particular clone in the immune hierarchy at the end of the primary response. Clones generated after the primary response are ordered according to decreasing cell number. This order is upset when there is a change of hierarchy occurring after the second challenge. Reprinted with permission from [11].



Following these simulations, we studied the effect of affinity for the heterologous peptide of the present memory repertoire on the clonal population changes after heterologous infection. We simulated memory T cell repertoires containing a given number of same-sized clones (e.g. 30 cells each). Each repertoire was designed to have a predetermined number of clones with a given affinity for the heterologous challenge. For example, VM were primed and programmed to show 10% high/90% low-affinity or 50% high/50% low-affinity clones toward the heterologous peptide in their memory repertoires (Figure 3(B)). Our results show that high-affinity clones have the highest probability of being immunodominant after challenge, although both the competition and the low initial representation can

thwart their chances. We also find that the repertoires may become more focused if the proportion of high-affinity cross-reactive clones before heterologous challenge is lower than the proportion of low-affinity clones.

Dissecting attrition

When a preformed memory repertoire is challenged by a heterologous set of epitopes, quantitative and qualitative changes occur [5]. If the challenging virus is antigenically distant but not completely foreign, some of the clones in the present memory repertoire will be cross-reactive, and some will not. The former will be then stimulated by the challenging epitopes and will initiate a clonal proliferation leading to a full response, while the latter could die due to the phenomena of either active or passive attrition. The distinction between active attrition and passive attrition is that cells may be directly killed by a factor such as IFN (active attrition) or they may fail to find a protective niche in the immune system due to competition with other cells (passive attrition).

The roles of passive and active attrition in shaping the T cell memory repertoire were simulated with IMMSIM by using the following hypothetical scenarios [12]:

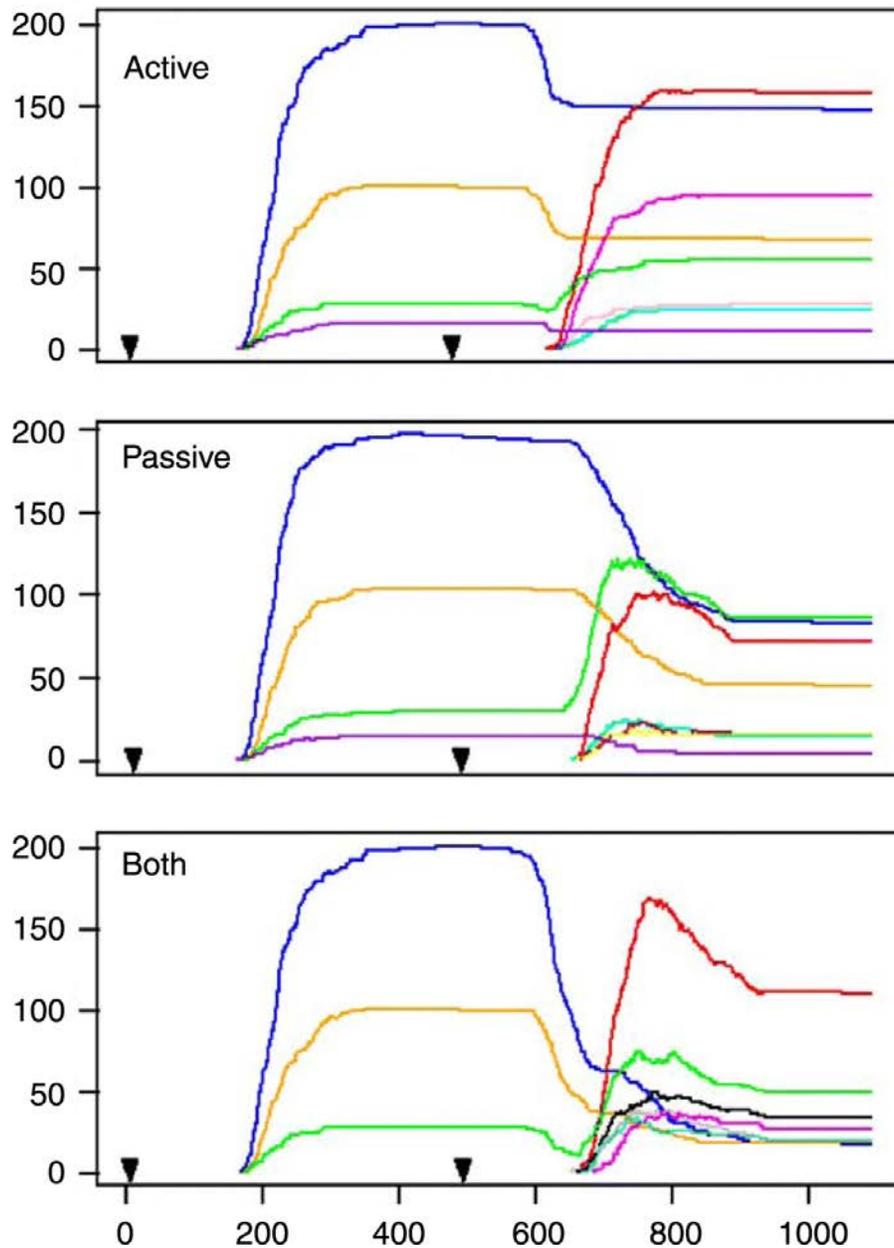
- 1 simulation of memory responses in the presence of active attrition only;
- 2 simulation of memory responses in the presence of passive attrition only; and
- 3 simulation of memory responses in the presence of both passive and active attrition.

As mentioned in the previous section, the two attrition mechanisms (“active” and “passive”) were simulated by introducing in the model decay routines acting stochastically on single cells. These routines are activated independently, either when a given total number of memory cells is reached during simulation (passive attrition), or by IFN secretion by infected cells (active attrition). IFN secreted by infected cells can diffuse locally and cause the death of non-stimulated cells, thus creating space for the growth of T cells specific for the heterologous challenge. On the contrary, protection against the cytokine is conferred to stimulated cells for a certain number of steps during the simulation.

Each simulation consists of a first challenge (V1) at time step (TS) 1, after which the primary response builds a memory pool consisting of several clones. After clearance of the primary infection, a second, cross-reactive challenge (V2) is delivered. Changes in the memory cell repertoire following cross-reactive challenge are monitored during the simulation (Figure 4). To study repeated secondary responses starting with the same memory formed during the primary infection (a simulation of their *in vivo* adoptive transfer technique), the same RN was used for the primary infection, while a different RN was allowed for each secondary infection. By enabling or disabling the different attrition mechanisms modeled, responses with active only, passive only or both active and passive attrition were obtained. Figure 4 shows a set of three representative experiments where T CD8 memory clones develop in these three conditions.

Figure 4. Mathematical modeling of memory T cell attrition by active, passive, or both mechanisms: TC memory clone dynamics during the primary and secondary response to cross-reacting viruses,

under three scenarios of attrition simulated by IMMSIM. Abscissa: TSs. Ordinate: TC memory clone cells numbers. Arrows at $TS = 1$ and $TS = 500$ indicate the time of inoculum of V1 (70 particles) and V2 (120 particles), respectively. V1 clearance is complete at $TS = \sim 200$ and V2 clearance at $TS = \sim 700$. Reprinted with permission from [7].



When active attrition is present there is a characteristic early decrease in all memory clones upon heterologous challenge. Cross-reacting clones show a typical “dip” before rising in the secondary response. On the contrary, the effects of passive attrition can be observed only later during the response and last for longer, causing a characteristic decrease of the secondary clones after their peak. These experiments also illustrate stimulation of new clones and a predominant presence of high-affinity

clones when active attrition only or both attrition modes are present. This is epitomized by the relative behavior of clones that have low affinity for V1 and medium affinity for V2 (green line, Figure 4) and of clones that have no affinity for V1 and high affinity for V2 (red line, Figure 4) in the three attrition modes. By contrast note the behavior of clones with high affinity for V1 but no affinity for V2 (blue and yellow, Figure 4) or clones with no affinity for V1 but higher medium affinity for V2 (purple, light blue and black, Figure 4).

In summary, we observed that after the challenge by the second virus, in the case of active attrition only and both active and passive attrition the growth of cross-reactive clones is impaired, allowing an opportunity for new clones to start off. The number of the latter is significantly larger in both these situations than with passive attrition only ($p < 0.01$, $n = 180$, Student's *t*-test). The pattern of clonal dominance is also influenced by the mode of attrition: extreme dominance of single clones is favored by active attrition, while co-dominance is more frequent with passive attrition, which exerts a flattening effect. Furthermore, the dispersion measured among the highest three clones of each simulation of the secondary response is significantly wider in active and both attrition than in passive attrition only ($n = 180$, $p < 0.01$ as calculated by two-tailed Student's *t*-test). Affinity edge is important to establish clonal dominance, but in these experiments it is only one of the factors; the success of the most affine clones is favored by active and both but not by passive attrition (Figure 4) [7].

Experimental studies in mice, as discussed in the preceding section, have strongly implicated cytokine-dependent active attrition as being a major player in memory T cell loss. It is noteworthy that this mathematical model predicts that this active attrition thwarts the expansion of cross-reactive T cells. This active attrition may therefore serve to temper the immunodominance that could be imposed by low-affinity but high-frequency cross-reactive T cells on the development of more effective high-affinity clones specific to well presented antigens.

Active attrition at work

Following these initial findings, we further investigated the role of active attrition on T CD8 memory [12]. Our initial hypothesis was that cell death by active attrition affected those members of the memory repertoire that were unable to bind antigen, that is that lymphocyte attrition following a heterologous challenge was selective and would directly favor the cross-reacting clones. However, the experimental data could not confirm any "protection" afforded to cross-reacting cells able to bind. In this case, simulations were used to challenge and explain the experimental results.

Computer simulations were used to determine the impact of virus-induced active attrition on the T cell response to a cross-reactive virus infection in VM bearing a partially cross-reactive pool of memory T cells. Simulations first showed that, in the absence of memory cell attrition, cross-reactive T cells would dominate a new T cell response and inhibit the emergence of a complex naive T cell response specific to a new pathogen. Following these initial results, we tested the hypothesis that early attrition of memory T cells would facilitate the development of a more diverse naive T cell response by simulating two scenarios:

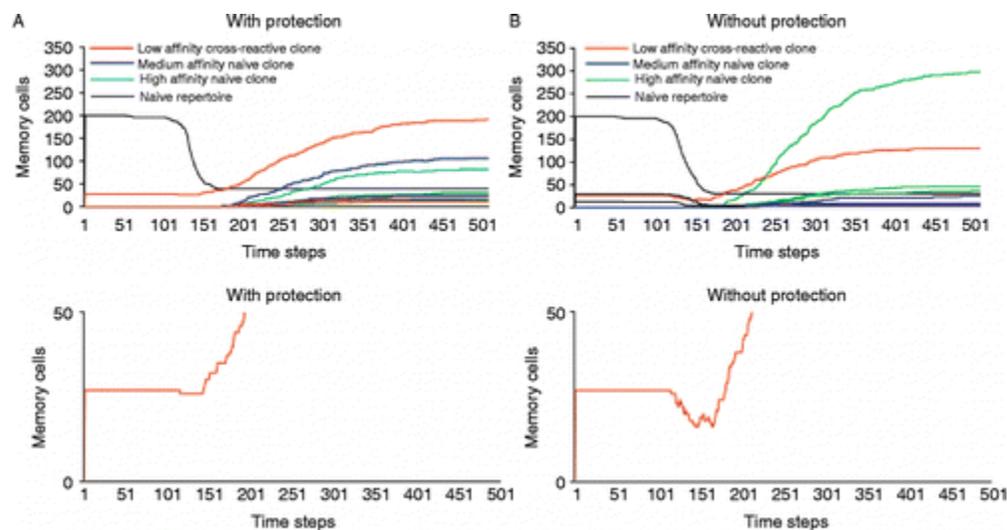
- 4 generalized cell death due to active attrition; and

5 selective cell death of non-stimulated memory clones due to active attrition.

In the first scenario, all clones (including the cross-reactive ones) undergo apoptosis upon infection. In the second scenario, after a successful interaction with an antigen-presenting cell, the stimulated memory T cell is protected from IFN type I-mediated death for a designated number of TSs, decided at the beginning of the simulation. Thus, during the heterologous virus infection, only the cross-reactive memory cell clones are protected from apoptosis, whereas the non-cross-reactive ones die. Simulations were conducted as follows.

A VM was challenged with a virus, which was completely cleared thanks to an effective primary immune response. The CD8 memory T cells formed in this primary response were transferred into different virtual recipients, and a second challenge with a partially cross-reactive virus was administered. Figure 5 shows a representative simulation depicting the two scenarios of generalized and selective cell death. In Figure 5(A), a low-affinity cross-reactive clone transferred from the donor is depicted in red. The y axis shows its frequency before infection.

Figure 5. Computer modeling indicates that the early apoptosis of memory cells allows for more diversity in arising T cell responses. A, Simulation depicting protection from active attrition, where a low-affinity, cross-reactive clone (red) dominates the immune response. B, Simulation depicting an absence of protection from active attrition, where a high-affinity naive clone (green) generates an immunodominant response. Plots below A and B enlarge the otherwise compressed plots for the low-affinity cross-reactive clone (red) to demonstrate the attrition without ligand-induced protection. The plots are representative of 30 repeated runs, (from [12] with permission).



When protection from active attrition is conferred to cross-reacting cells, this low-affinity clone can dominate the immune response against high- and medium-affinity clones (green and blue) originating at low frequency from the naive memory pool of the recipient (Figure 5(A)). However, if this low-affinity cross-reactive clone undergoes apoptosis (Figure 5(B), in the case of generalized cell death), an

immunodominant response can be generated by higher-affinity clones (i.e. green) from the naïve repertoire.

In conclusion, by observing side-by-side the mechanisms of generalized and selective attrition we could predict that early generalized apoptosis of memory cells allows for more diversity in newly arising T cell responses. These results indicate that if a situation arose whereby cross-reactive memory cells did not undergo active attrition, they would zealously dominate an immune response. This prediction has recently been confirmed by *in vivo* data. Recently, it has been shown that more than one-year old mice undergo considerably less IFN-induced active attrition than young mice. Consistently with our results, when immune mice were challenged with a heterologous but cross-reactive virus, cross-reactive memory clones dominated the secondary response far more in old with respect to young mice, due to the lower level of active attrition.

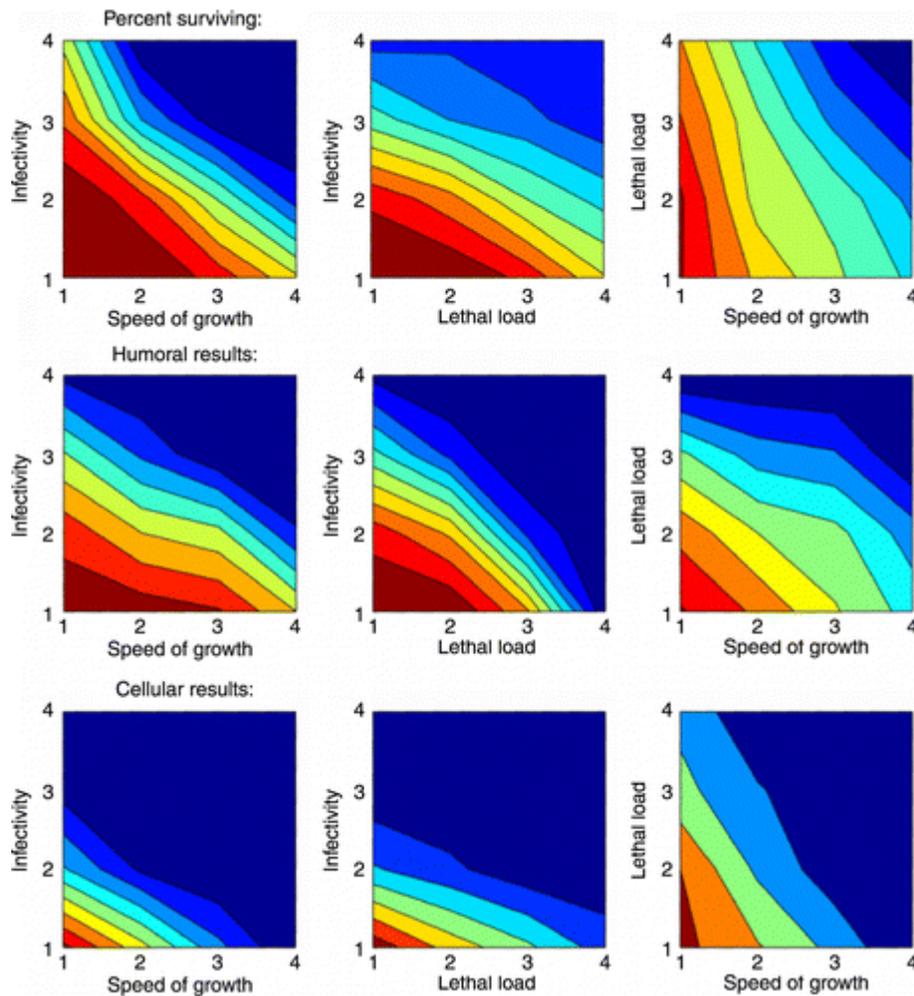
Competition and cooperation between the humoral and cellular branch of the immune system: the case of split cross-reactivity

The engagement of both branches of the immune system is necessary in some responses, useful in many and disposable in few cases, in order to clear infections. The presence of two semi independent immune systems in vertebrates is clearly the evolutionary response to the development of sub-cellular infectants, and to their presence - at periods - outside and inside cells of the organism: when outside the viruses are not attainable by cellular responses, while when inside they are not reachable by circulating Abs [1,2].

Consequently, to conquer viral infection, two different machineries are required at the same time, even if the relative importance of the two varies along a large spectrum [14]. In one of its first applications, IMMSIM was used to simulate the host response to 64 randomly assorted viruses with different physiological characteristics (infectivity, speed of duplication, and lethal load) but antigenically identical in order to explore cooperation and competition between the two branches of the immune system. We compared the mortality of "VM" in cases where both or only one of the branches of the immune system was allowed to take part in the response to any given virus.

The results showed that certain combinations of viral replication speed, degree of infectivity, and lethality make the infection more sensitive to either humoral or cellular responses, while the cooperation of the two branches always showed the highest clearing power (Figure 6). In addition, signs of competition for antigens between the two branches emerged in several simulations and allowed to formulate predictions in this direction [15].

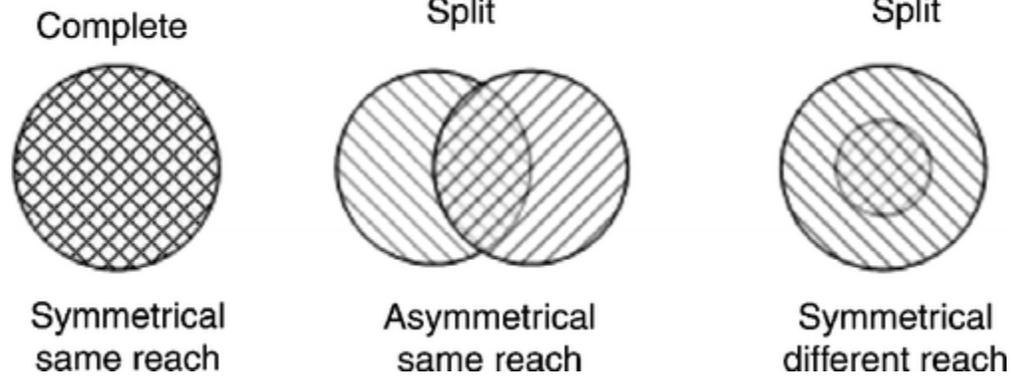
Figure 6. Contour plots of survival frequency as a function of a range of two viral physiological parameters at a time. Each plot is coded in the same way: the dark blue areas indicate 100% "VM" dead, while the dark red area indicates 100% survival rate. Each change in shade from dark blue to dark red represents a 10% increment in survival rate. The three graphs in each row show (from left to right) plots of survival rates depending on infectivity and speed of growth, lethal load and infectivity, and lethal load and speed of growth. The top row shows results obtained when both branches of the immune system were acting, the middle one shows results obtained with the humoral branch only, while the bottom one shows results obtained using the cellular branch only. Reprinted with permission from[14].



In summary, these results documented the selective advantage of the cooperation between cellular and humoral defence, but predicted that also, in varying degrees, a competition for resources between cellular and humoral response should be expected. These results are confirmed by our most recent systematic simulation of cross-reactivity [15] involving both branches of the immune system. In these simulations we propose two scenarios (Figure 7), one in which cross-reactions are represented equally in the two branches and the other where cross-reactions can take place in only one of the branches.

Figure 7. Cartoon to represent hypothetical overlaps of humoral and cellular cross-reacting repertoires. Only the central and the right drawing are compatible with split cross-reactivity. Upward diagonal hatch mark: humoral response; downward diagonal hatch mark: cellular response. Reprinted with permission from [15].

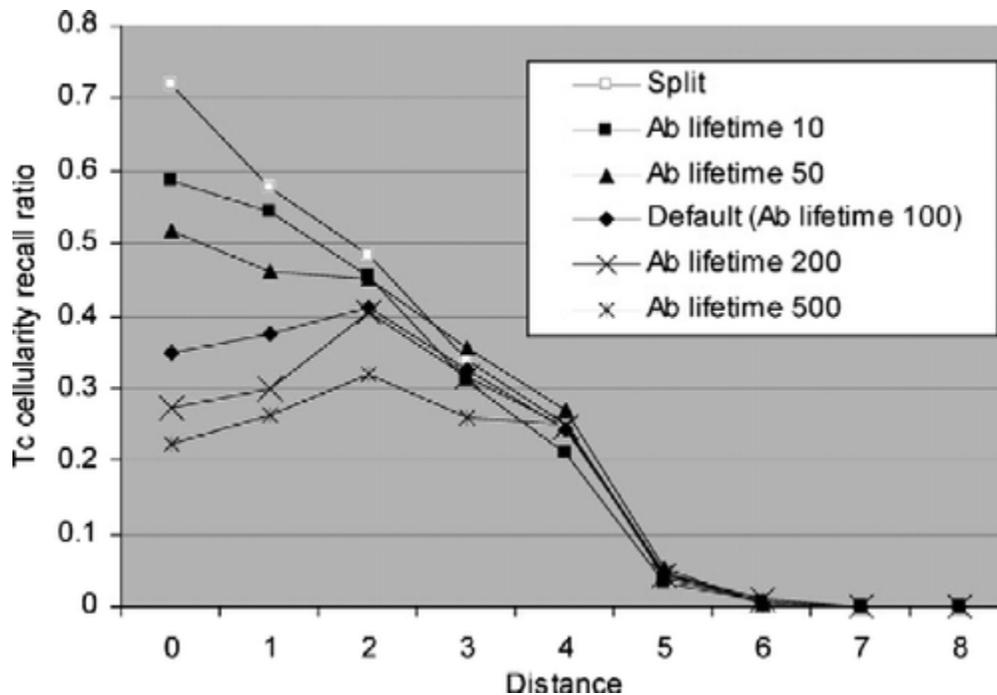
Theoretical overlap among Humoral and Cellular repertoire*



The second scenario is consistent with the findings of heterologous immunity, where serologically unrelated viruses cross react at the T cell level. We showed that with viruses bearing physiological characteristics that make them sensitive to the cellular branch, the presence of a cross-reacting humoral response is mostly a nuisance as it competes for antigen without contributing decisively to the defence (Figure 7). In this case, we simulated either homologous or heterologous (with systematically increasing antigenic distance, D) primary and secondary infections.

In Figure 8, we show the fraction of primed memory cells that are stimulated by the secondary challenge. As expected, this fraction is largest (0.7) at $D = 0$ and declines to 0 for antigenically different pathogens. Also, the largest number of recalled memory cells is obtained when only the cellular branch is allowed to participate in the response (a situation labeled "split" response), but is reduced when both responses are allowed. This reduction in the number of recalled memory cells becomes progressively more severe when the strength of the humoral response is enhanced artificially by elongating the lifetime of circulating Abs (Figure 8). Among the cases where the cellular response alone can successfully contrast the infection are those where heterologous immunity was initially described [3].

Figure 8. This figure shows the fraction of recalled T CD8 memory cells in conditions of split cross-reactivity, with and without artificial reduction (to 10, 50) or extension (to 200, 500) of Abs half-lifetime. ANOVA is conducted for distance 0, 1, 2, 3, 4 and the F -value > 3.35. Clear squares: split; black squares: Ab lifetime 10; black triangle: Ab lifetime 50; black diamond: the default Ab lifetime 100; crosses: Ab lifetime 200; stars: Ab lifetime 500. Reprinted with permission from [15].



Discussion

In this review, we describe the joint interdisciplinary efforts of two research groups to investigate several aspects of the immune response against viruses using both computational and experimental methods. Our collaborative work explored the role of the two branches of the immune system in both homologous and heterologous secondary infections, with particular attention to the role of T CD8 cross-reactive memory and its changes upon secondary cross-reactive challenges. Some of the many advantages and possible limitations of computational modeling of the immune system are highlighted throughout this manuscript.

Some of the limitations of computational simulations arise from the need for simplification, which to a varying extent can prevent the modeling from capturing the system's complexity. This is well exemplified in the study of cooperation and competition between the two branches of the immune system with IMMSIM: while the competition revealed by simulations is likely to happen also *in vivo*, it might be limiting to attribute it solely to competition for antigen as the model suggests [14].

However, while extreme simplification is a potential liability of mathematical modeling, it should be noted that in this case simulations allowed to explore scenarios very difficult to obtain *in vivo*, such as allowing only either one of the branches to participate in the immune response and to compare results in the case when both branches were active; or in another case, to manipulate the time of persistence of antibody in the circulation to better evaluate their competitive valence [15].

A similar approach was used to study the mechanisms of attrition, when simulations were performed with either active or passive attrition or both mechanisms being present, thus allowing to dissect the contribution of each one in shaping the T CD8 memory repertoire following secondary infections [11,12]. A similar approach allowed investigating more deeply the mechanisms of active

attrition, and predicted, in agreement with experimental results, that a cytokine-mediated non-selective loss of memory cells happens at the beginning of secondary infections [12].

In other cases, we have shown how surrogate of experimental techniques can be used in computational simulations to recreate specific situations, such as the adaptation of the adoptive transfer technique to *in silico* experiments to control the composition (in terms of number and size of clones and percentage of low- and high-affinity clones) of the T CD8 memory repertoire, to study systematically the mechanisms of active attrition. In addition, we have shown how the flexibility allowed by computational modeling allowed us to manipulate the theoretical size of the B and T cells repertoires, thus recreating in a minimalistic setting the variability among different individuals in terms of specificities in the naive repertoire and therefore in quality of the immune response to different pathogens. Furthermore, we were able to systematically study the mechanisms of heterologous immunity by manipulating the antigenic distance between cross-reacting epitopes, their respective sensitivity to the cellular and humoral response, and the viral physiological characteristics.

In conclusion, computational modeling of the immune system can be interesting and useful when it helps the system's complexity become more manageable and understandable, or if it can present hypotheses or produce new ideas that may be tested *in vivo*. For this to become standard practice, the first requirement is to engage in full collaborative efforts crossing the lines of traditional disciplines, where the collaborators fill roles with an equivalent level and quality of work. The second and equally central requirement is to be able to evaluate the modeler's contribution: is the nature of the simulations adequate to represent the biological problem investigated? Are the predictions focusing on fundamental problems?

Unluckily, most of these questions may only be raised after the fact, and preferably, based on a series of simulations, rather than on single assignments. The present publication tries to facilitate this point, for the case of the model IMMSIM, by gathering work performed by researcher at NYU and UMass over a period of many years, and displaying them in a readable form. We hope that accessing these works will facilitate understanding, elicit motivated criticism, and trigger fruitful discussion on the role of computational modeling in immunology.

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