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Systematic simulation of cross-reactivity predicts ambiguity in Tk memory: It may save lives of the infected, but limits specificities vital for further responses

Yanthe E. Pearson¹, Yiming Cheng^{1,3}, Liisa K. Selin⁴, Roberto Puzone², and Franco Celada¹

¹Division of Rheumatology, Department of Medicine, New York University School of Medicine, New York, NY, USA ²IST, National Institute for Cancer Research, Genova, Italy ³Philip Morris International, Neuchatel, Switzerland ⁴Department of Pathology, University of Massachusetts Medical School, Worcester, MA, USA

Abstract

The present study uses the agent-based model IMMSIM to simulate immune responses to a viral infection, with a focus on the impact of preformed memory (homologous and heterologous) on the quality and the efficacy of the response. The *in machina* results confirm the observed thwarting of new, naïve responses exerted by cross-reacting memory, but they also reveal that the competitive inhibition is made possible by the different time frame used by the primary and the secondary response, a well-known fact, epitomized by the interval of about 75 time steps between their peaks. This novel finding justifies the depression of naïve responses and the long-term consequences it could bring about and the role of memory as a player in a *survival of the fittest* game.

Keywords

Cross-reacting memory; dynamic competition; clonal dominance

Introduction

Cross-reaction is the compromise by which specific immunity can cover the enormous number of potential antigenic epitopes with an objectively large (but relatively small) repertoire of receptors/paratopes. It means that any receptor, besides a theoretical “best fitting” epitope, can alternatively accommodate a number of similar epitopes, even if their steric, electrostatic, and hydrophobic characteristics are suboptimal. Reciprocally, any epitope can be recognized by a number of different paratopes/receptors. The highest attraction (or affinity) will designate the best-fitting combination, while the affinity of cross-reacting bonds will be up to several orders of magnitude lower. In the humoral response only small determinants (groups 3–5 amino acids) on the surface of the antigen (Ag) and directly accessible by the B-cell receptor (BCR) or antibody (Ab) paratope serve as epitopes, and the probability of binding is governed by the affinity between the two clusters.

In the cellular response, e.g. against virus, one additional player is required, and adds to the complexity: the epitope must be “created” by a processing event which takes place in antigen presenting cell (APC) cells, epitomized by the dendritic cell: the viral proteins are digested and reduced to small peptides, of which only those of a narrow size range and equipped with certain anchoring groups will be associated to one of the several major histocompatibility complex (MHC) receptors, thus forming the epitopes. Each response will use the same epitope both in the phase of activation of T_H cells, and in the eventual binding and killing process performed by the mature effector cell.

Because the epitope is exhibited on the surface of the virus infected cell, it is the infected cell that is killed, while the virus destruction may occur as an indirect consequence. The higher complexity of the T-cell response and the threesome binding of MHC, peptide and T-cell receptor (TCR) may justify the existence of larger repertoires and/or a higher probability of cross-reaction among “foreign” pathogens, compared with the humoral response. This is suggested by experimental findings of what we called “split cross-reactivity” [1], where two viruses may result to be totally foreign in a serological test and yet cross-reacting in a cellular response.

Cross-antigenicity among foreign (heterologous) viruses is the fundamental finding of Selin and Welsh [2], who described the consequent heterologous immunity as a fundamental paradigm to understand the defense against infection. The first casualty of heterologous immunity was the (classic) idea that memory is an immobile; long lasting asset that will be activated even decades after the original stimulation. In the new paradigm, memory is defined by repertoire of clones selected by the original priming whose relative dominance is dictated by the number of lymphocytes representing each clone. This hierarchic order is not permanent, but is exposed to the effects of new Ag encounters, e.g. during any new infection, where each cross-reacting clone will be stimulated to grow in number and dominance while the others will be thwarted by active attrition and lose status. This shatters hierarchy and produces a “plastic” memory. This situation can be illustrated by the following statements:

1. nobody is naïve: the probability (for mice, and even more, men) to harbor memory clones cross-reacting with a random infectant grows with lifetime;
2. nobody is homologous: the evolutionary tendency of escaping dangers by utilizing random mutations is typical of all pathogens and applies also when the danger is the immune response: thus, the probability of a vaccinated organism to meet an identical Ag twice is dwindling; and
3. everybody is heterologous is the conclusion of this syllogism and points toward the importance of cross-reacting in the present paradigm.

Materials and methods

The simulator

The IMMSIM model considers discrete events (e.g. in the immune response, the encounters between single cell and molecules) inside a quantized “lattice” space. It works from these events to delineate the whole simulation, which evolves in discrete time steps (ts). In its simple form, the model consists of a 15×16 bidimensional grid hosting different entities in variable amounts. These entities are cells, e.g. T and B lymphocytes, APC, epithelial cells (EC), etc., and molecules such as Abs, Ags, and cytokines; because of the emphasis on interactions, it is easiest to consider the IMMSIM space as a virtual immune system (IS) organ, such as a lymph node. The entities are selected depending on the aspect of the immune response to be simulated and their quantity can be decided at the beginning of each

simulation. Entities are represented by data structures storing tuneable working properties (e.g. interaction strength, clonal expansion, death, and diffusion) and present individual status (e.g. position in the grid, age, and activation state).

The agents

Most agents can diffuse within the grid; interactions between individual agents obey probabilistic rules, and are allowed only if they “meet” within a site in the grid. Any interaction between two entities is limited to a single successful one (if any) at each t_s of simulation; the t_s is the common pacer in all the interactions.

Because the fastest cell divisions take one t_s , the latter can be considered equivalent to 6 h. Ag and Ab specific properties, and the clonal characteristics of the cells, are represented by assigning to each single entity one or more individual “receptors” in the form of an N -bit binary string. All specific interaction rules depend upon the “matching” of the two interacting actors’ receptors; the rule is a function of the binary distance of the respective N -bit binary string. Any single interaction usually results in a discrete alternative (to die or not, activate or not, divide or not, diffuse or not, etc.) that is stochastically decided according to the probability calculated by the interaction rule. What happens at each step depends upon the contents and conditions of each site, yielding a system that can be described as a stochastic agent-based discrete model.

The model incorporates many of the consensus facts of today’s immunological knowledge, e.g. the diversity of specific elements [3], MHC restriction [4], clonal selection by Ag affinity [5], thymic education of T cells [6], Ag processing and presentation [7], cell–cell cooperation [8], and maturation of the response and memory [9], all tuned in the model to balance the responses in the minimalistic scale (compared to biology) of the simulations. As the stochastic behaviors in the model is based on a random number generator, when repeating a run with the same set-up but different initial random “seed”, it will result in a number of virtual mice (VM).

The cells

Antigen presenting cell—APC has MHC class I (MHC1) and class II (MHC2) receptors, is not specific and has low affinity for all Ags, viruses. When in proximity of a source of DANGER signal, becomes activated and is able to activate any T-helper (Th) cell and any TCR specifically. B cells act as APC, since they process and present Ag reacting with its BCR, but are not able to activate Th *ex novo*.

B—It has specific BCR (recognizing Ag epitope) and MHC2. When it binds Ag the latter is internalized and processed, and the hidden peptide is mounted on MHC2 receptor. The combination of a part of peptide and MHC constitutes a T-epitope that may be recognized and bound by a Th2 cell’s TCR. For this interaction to be effective, the Th2 must have been previously activated by an active APC.

Th cell type 1—Th1 has specific TCR capable to bind to a APC’s MHC presenting a peptide. This causes the APC to activate a Cytotoxic T lymphocyte (Tc) if the two T cells bind to the APC at the same time or within a given time lapse.

Th cell type 2—Th2 has specific TCR capable to bind to a B-cell’s MHC presenting a peptide. This constitutes the signal for triggering the B and Th-cell’s activation process (simulating the lymphokine secretion and activation effect).

Cytotoxic T lymphocyte—T_c has specific TCR. When naïve, it will be activated if the TCR binds specifically to an APC's MHC1 presenting a peptide. When activated or in memory state, it will be able to bind by its TCR to any specific loaded MHC on virus-infected EC. This TCR binding causes the death of the EC and the elimination of all virus particles it contains.

Epithelial cell—EC is the example of body cell not belonging to the IS. As such, it has only MHC1. If infected by virus, it signals the presence of an invader by displaying a viral peptide on the MHC1. If the virus completes its proliferation inside the cell and reaches its critical multiplicity, EC bursts open, dies and frees the viral particles into the environment and diffuses a DANGER signal.

The molecules

Antibodies—Ab is identical to the BCR of the B cells that secretes it when it becomes a Plasma cell. Diffuses. Binds epitope by affinity proportional to bit complementarities. If it binds to a free virus, the latter is killed, cannot penetrate into cells, not even if they are infected.

Antigens—Ag is composed by two binary strings (more epitopes, or more peptides can be added for *ad hoc* experiments). One string (epitope) is accessible by specific BCR and Abs. The other (peptide) is not visible, and becomes accessible only when a B-cell or an APC bind the Ag, internalizes it and displays it on MHC2 after processing. If Ag amount exceeds the parameter “Death load”, the simulation stops, declaring the death of the host.

Virus—Virus is an Ag with the added capabilities to penetrate ECs and begin duplication when inside. Each virus has specific epitopes and peptides. In addition, it has one degree, low, moderate, high or very high, of the three properties that define its lifestyle: (a) I, infectivity (probability of penetrating into an EC in the same site); (b) S, speed (number of duplications/ts); and (c), L, lethality (multiplicity of virus particles that will cause the host EC to burst).

Interferon—Interferon (IFN) represents a cytokine and is the effector of active attrition. A molecule of nondefined structure is selected in controlled amounts, has controlled life time, and diffuses. Its presence causes controlled apoptosis in memory cells present in the site.

Danger—Danger is a virtual cytokine and the effector of nonspecific APC activation. Selected in controlled amounts in the site where virus-caused cell death occurs and has controlled life time and can diffuse.

Response to viral infection

Whether the infection is cured or becomes persistent or even kills the virtual mouse depends on the virus dose and the quality (determined by its combination of infectivity, multiplicity, and lethal load levels). These also determine whether the IS success requires the cooperation of both the cellular and the humoral branch, as has been shown in several occasions [10].

The parameter values in Table I are used whenever the program applies the “sequence of events” described above. The result is the viral infection of a VM followed by a full immune response. Runs are governed by random numbers generated by the computer, since all events, such as cell–cell and cell–molecule encounters, are probabilistic. A rerun with a different set of random numbers results in a different VM and a different response, even if the same list of default values is used. The default values are based on consensus among immunologists, but are not “real”, as they reflect the model's simplification/minimization of

the real response. They cannot be singled out, since they were selected in relation to each other and balanced by *in machina* experiments.

Rationale

Systematic probe of cross-reaction

During the ongoing collaboration with the virologists of UMass we have used IMMSIM, and establish an agent-based model to simulate experimental findings [10]. Very recently we have tried it in an avant-garde, exploratory fashion, to confront the biologists with data generated *in machina* after feeding it with real backgrounds: the aim was to generate hypotheses about the impact of cross-reactivity on the efficiency of secondary response [1].

Relying on the flexibility of the model, we used a version that can generate a theoretical diversity of 68,000 epitopes and paratopes, and challenged VM endowed with 2500 clones for each of the effectors or helper lymphocytes, with two stimulations separated by 600 ts. The antigenic distances between the priming and the challenging virus ranged from 0 to 7; each mouse could recognize Ags binding at 0, 1, 2, and 3 mismatches. Two Ags could theoretically be engaged in cross-reaction when differing by five steps or less. Our aim was to measure the impact of antigenic distance between a standard immunization and a standard challenge on the quality and efficiency of the final response. Responses to eight types of stimulation were monitored and compared with each other in terms of speed, cellularity, and affinity to the second Ag of the cellular and (in certain cases) of the humoral response, while their degree of efficiency was deduced by their effect on the infection, the timing and multiplicity of the viral peak, and the time to clearance.

The cross-reactive responses were produced “in a matrix” by exposing ~10,000 VM randomly to two out of five viruses differing from each other by 0, 1, 2, 3, 4, 5, 6, and 7 binary bits; the infections took place at $T = 0$ and $T = 600$, and the runs lasted 1000 Ts. The data emerging and their variations in time from the eight treatments were analyzed according to the following parameters: number of particles, number of B cells, Ab titer, number of Tk cells, number of Tk1 and Tk2 cells, affinity of each type of lymphocytes, and present number of different clones of Tk, Th1, Th2, and B cells (repertoire size). This matrix experiment was repeated in the absence of the humoral (secondary?) response. To mimic the experimental finding of the UMass group of a “split” cross-reaction, i.e. limited to the cellular response.

The findings

The success of the virus—The impact of the systematic increase of the antigenic distance between the primary and the challenging virus results in a nonlinear decrease of efficiency of the response, in terms of capacity to thwart the success of the infecting virus. This results in a progressively larger total multiplicity of the virus, as shown in Figure 1.

Cellularity and affinity of the response—The quality of the response showed a biphasic behavior. In other words, the strength of the Tk response in terms of number of cells multiplied ($\times$) by their affinity toward the challenging virus after the second infection is highest at $D = 1$, where the second virus is identical to the first. We can assume that the entire response is a memory response. By increasing step number (the distance between the two viruses) the response is sustained by cross-reacting memory cells and by naïve cells producing a primary response to the new Ag. The impact of the cross-reacting memory cells decreases stepwise while the participation of the naïve cells increases gradually, peaking after $D = 5$, which is the limit after which no cross-reaction can exist. From the virus data of Figure 1, we expected a smooth decline from the secondary level strength and of $D = 0$ to

the level of a primary response. The results however, showed a dip at intermediate distances 2, 3, and 4. Here, we illustrate this finding in Figure 2a,b,c, from the original study of “quality” vs. distance.

Figure 3 presents for each distance a histogram of the cells separated in the four effective affinity levels toward the challenging virus (when 1, 2, 3, and 4 correspond to binding with 3, 2, 1, and 0 mismatches), note that the occurrence of a 0 mismatch between paratope and epitope is an extremely rare occurrence in a 16-bit setting, and therefore 1-mismatch binding represents the highest affinity attainable. The highest frequency (peak) of each histogram falls successively from affinity levels 3, 2, 1, 2, 2, 3, 3, 3 confirming the sinusoidal movement in Figure 2.

Results

Dissection of the response

The response to the second virus, already divided in eight types according to the antigenic distance of the two viruses injected was analyzed by dissection, separating (a) the memory cells present at the moment of the second injection, (b) the memory and naïve response at its peak, and (c) the naïve (primary response).

The initial memory load—Memory cells are formed during the primary response, a number remains (about 250) at time 600 or 800, which is time 0 of the secondary response (Figure 4a), since each $D=0$ to $D=7$ experiment was pruned with a different virus, their memory repertoires are different and only memory cells of the experiments $D=0$ to $D=5$ can recognize the challenging virus, and the affinity decreases with distance as shown when the memory cells are separated according to their affinity level (Figure 4b). The strength of these responses (affinity weighted by the cell numbers) decreases dramatically, as shown in Figure 4c.

The response to the challenge virus—This response is measured in terms of peak numbers of total Tk cells reached after the infection by the challenging virus (Figure 5a) and by the breakdown of the Tk cells according to their level of affinity (Figure 5b). The responding Tk cells are almost exclusively memory cells performing a secondary response in $D=0$, cross-reacting memory cells with some participation of naïve responses in the intermediate distances, and exclusively naïve response maintains a primary response in $D=6$ and $D=7$.

The naïve (primary) response—The exclusively primary response to the second virus is isolated in the panels of Figure 6. These figures practically repeat the primary response of the far D 's of Figure 5 and underline the finding that the primary response is prevented from developing in the presence of the secondary response (even when the secondary response is of low efficiency because of far cross-reactivity). Figures 5 and 6 show a dramatic “size difference” of the primary responses in the absence or presence of a comparatively small number of memory responding cells. To complete the picture and to propose a credible mechanism for this competition we have to analyze the impact of time and of Ag availability.

The time factor—The separation of memory response from naïve response (see Figures 3–5) shows that the latter is severely thwarted at all “antigenic distances”, where memory is fully or cross-reactively able to mount a response. Since the engine of the competition is Ag, to fully understand the nature of the competition between memory and naïve response it is necessary to analyze the dynamics of Ag production. Ag availability, in an active infection

increases with time, and the amount of Ag determines the size of the response. On the other side, the duration of the infection is inversely proportional to the size/strength of the response. The speed of the response (measured from the time it takes to limit and clear the infection) is proportional to the number of cells initially stimulated, and in this case to the number of memory cells which are present at the starting point.

Figure 7 presents the data of virus life corresponding to the various antigenic distances between first and second infection; in Figure 7a, they are plotted with differential Y values, while in Figure 7b with the same Y value, in order to demonstrate both the shape of all curves and the relative site of total virus produced before the clearing of the infection. The virus peak for $D=0$ occurs at 120 ts; at this time the memory response has reached its full development and will begin to cause the decline of the virus.

The existence of a second, lower peak at about $T=200$ suggests that new cells may participate in clearing the infection. The early peak is visible in all conditions where cross-reactive memory exists, $D=1$ to $D=4$, but the peak at $t=200$ is the most prominent one. At $D=5$ to $D=7$ the late peak is the only one, indicating that the infection progresses undisturbed until $t=200$ before being limited and cleared. Figure 7b highlights the quantitative difference in virus produced in the $D=0$ to $D=7$, and shows that in the homologous response the early development of the memory response drastically reduces the production of virus, which instead reaches its highest peak in the absence of cross-reactive memory.

This fully explains why the development of the primary naïve response at high levels is not permitted whenever the memory response, albeit cross-reacting is present, and pre-empts, the production of Ag. In other words, the competition between memory and naïve response occurs when the latter has not yet begun to form. This finding effectively reconciles the data of Figures 1 and 2. There are two more considerations, which may in part depend on the parameter setup of the IMMSIM-C version used.

One is that affinity maturation is enhanced by the size (number of cells involved) of the responses and this explains the higher average affinity reached in the full-blown naïve response compared with the intermediate cases. The second is that the model does not incorporate a higher multiplication rate for memory cells, compared with naïve cells; therefore, the faster deployment of the memory response must be prompted by the presence at time 0 of multiple copies of specific memory cells ready to encounter the MHC-presented epitopes and mount the response. From the data shown previously, in Figure 4a, we can infer that each clonotype in the memory may be represented by more than 10 effector cells. The time of the virus peak and the Tk response peak are plotted in Figure 8.

The two curves run roughly parallel from $D=4$ to $D=8$, but the T success is proportionally larger than the viral success at $D=0$ to $D=3$. This observation is reiterated by plotting the ratio of Tk and virus success for each distance. The shape of the curve ratio could be justified by a (near) efficient stimulation of the memory cells compared with the naïve ones. Again, since there are no provisions enacted for a differential mechanism of stimulation of Tk cells in the model, we tend to attribute this finding to a higher number of specific cells present at $T=0$, the memory cells.

Figure 9a is a log plot of Ag and Tk success (peak $\times$ time of persistence) vs. antigenic distance between pruning and challenging virus. Figure 9b is the ratio between Tk and Ag success and shows a decrease of relative efficiency of the memory response with increasing antigenic distance.

The total “success” measured by multiplying the peak value by the time of actual deployment of virus and of response is plotted in Figure 9a (legend: color codes for Ag and Tk success). The two logarithmic functions start widely apart at $D = 0$ and converge gradually until $D = 4$, where they become roughly parallel, indicating that an equilibrium exists between the size of the response and the size of the viral infection. This equilibrium is characterized by a log distance = 1 between the curves.

At the maximum diversion ($D = 0$), the success of the virus is 1, 5 log units smaller than its highest level (marking the difference in efficacy of the secondary response and the primary response), while the distance between the two curves is highest and measures 2 log units, because of a relative increase of the Tk success compared to that of the naïve response. One could also interpret the relatively higher success of memory response as due to a better utilization of Ag when the density of specific cells is higher, resulting in a larger overflow of Tk cells over the level strictly necessary to curb the infection. This mechanism is also supported by the longer “time to peak” shown by the memory responses in Figure 8. The third curve in Figure 9 plots the ratio between the Tk success and the viral success, and shows even more clearly the “premium” effect enjoyed by memory responses.

Heterologous response and viral lethality

Scheme of simulation—The grid contains 48 groups of VM, 1A to 8F, each formed by 80 individuals totaling 3840 VM. Each group receives a priming virus at time 0. The eight different priming viruses are shown on the ordinate, defined by their speed of multiplication (in green) and their antigenic distance with respect to the challenge (in red). The six different viruses used for the challenge at time 600 are defined on the abscissa. They are all identical antigenically, but vary in multiplication speed from 1.05 to 1.30; these factors represent the increase of virus particles at each ts.

Memory and survival—The virtual virus used in our simulations is defined by its specificity (epitope + peptide) and by three behavioral parameters: infectivity, lethal load, and speed of duplication. The latter is critical in the “race” with the immune system. To analyze the response, we have used—up to here—slow viruses (speed = 1.05), which are eventually cleared also by naïve VM. To follow up on the importance of dynamic factors in the infection/response we have repeated the systematic study of cross-reactivity, but introducing the variable of “virus speed”.

In essence, each group of VM immunized by homologous or stepwise removed cross-reaction (“live/attenuated virus”) was subdivided and challenged with six “speed variants” of virus, antigenically identical. A matrix scheme with the actual specifications is added where 48 VM groups from 1A to 8F are listed, each group composed by 80 VM. The total number of VM is 3840 (shown in the previous Scheme 1).

The first effect of increasing the speed of virus is an increasing proportion of deaths. Nonimmunized VM (those primed with virus of $D + 6$ and 7) have the lowest survival rate, while those primed with $D = 0$ have the highest, with over 60% at virus speed 4. The cross-reacting groups, primed with $D = 1, 2$, and 3 all show a life-saving effect of memory, albeit progressively decreasing (See Figure 10a,b). The study of the impact of viral speed of duplication in Figure 10 and Scheme 2 shows that virus with speed 1 can be cleared in over 80% of the cases, even by a purely primary response, while infection with speed 2 virus is overcome in about 25% of the mice.

Speed 3 serves as a divide between naïve and memory (no VM with only naïve response survives), while memory exercises life-saving effects, the stronger the survival rate the smaller the antigenic distance: from 100% at $D = 0$ to 10% at $D = 4$. At the lowest speed,

substantial survival is obtained not only by memory but also by a totally naïve response, reading a survival rate over 80%, while speed 3 serves as a divide between naïve (no VM with only naïve cells survives) and memory (a small effect is felt even at $D = 4$) which exercises stronger life-saving the smaller the antigenic difference. Speed 2 allows, naturally, a stronger life-saving effect by memory, and also a significant effect by the naïve responders.

The next analysis regards the “quality” of VM death. Death is determined in the model by the destruction of 50% of the ECs by the bursting virus or by the Tk cell killing infected host cells, and the speed of death is the time it occurs after the challenge. The results show a correlation of speed of death and the duplication speed of the virus. In borderline groups, where the memory is a life-saving factor, it is not the speed of the death that is decreased but the fraction of survivors that is increased. These findings are shown graphically in Figure 11.

Discussion

The central finding of our systematic modeling of cross-reactivity in a response to viral infection [1] is that the presence of memory (in particular Tk memory) either specific or cross-reactive has a double significance: (a) it helps in the clearing of infections, even life saving if the virus is lethal and (b) it hinders naïve cells from developing a full fledged, better quality primary response. The phenomenon has some relation with the original antigenic sin [11] described in the response against influenza, when a new infection elicits Abs specific for epitopes of a strain responsible for a prior infection. Since we were not trying to simulate the phenomenon, the novel *in machina* finding has the role of a validation of IMMSIM and inserts the concept of antigenic sin, and its proposed mechanism becomes a central tenet of cross-reactive memory.

However, this must be confirmed by the experimentalists; in the cooperation with the UMass virologists, while the task of the modelers is to formulate a detailed hypothesis to test. Thus in the present study we try to show the mechanism of the competition. The original observation was made when plotting the peak “quality” of the response in eight variations of “primary + secondary responses” where the first and second infecting virus VM were separated by increasing antigenic distance, from 0 to 7: the graph, instead of a progressive decrease of weighted affinity from the level of a specific secondary response to the level of any primary response showed a biphasic shape, with a low-strength pit in the middle distances.

Could thermodynamics be wrong?

How can cells, necessarily of low affinity because primed by a different virus, out-compete potentially better cells? Could “mass action” be invoked, since the memory clonotypes were represented by comparatively high numbers of cells? This idea has two weaknesses: it imagines an open competition (with all competitors granted the same chances to contact Ag), and a static one, like the encounter of “dead” molecules such as two types of Abs mixed with an Ag in a test tube. Instead, in the real world and in the simulation, cells and virus are alive and can multiply or die, and their meeting takes place in a flux.

Time dimension

IMMSIM simulates this flux as 3D (bidimensional space + time), where the players enter at different moments while paratopes and presented epitopes confront and bind each other with probabilities proportional to their mutual affinity. At time 0 when Virus 2 is injected, memory cells are already there, an average of 250/VM (compared with the single digits of

naïve cells, an overwhelming number). They are the result of the growth, differentiation, and decline of the primary response.

Because each VM has approximately 20 cells recognizing any single epitope we assume that the memory represents the clones grown out of one half of the possible target cells (that is, about 10). If so, each memory clonotype would count an average of 20 cells, not considering the impact of clonal dominance. Time 0 is the beginning of life for 20 virus particles that enter and start to multiply in ECs. Some time later ($t_s = 40$) 80 new particles burst out, some are captured by APC processed, and their epitopes are associated with MHC and presented to the Tk.

This is the real moment of competition, perhaps around $t_s = 50$, when the three players of the competition, the memory clones, the naïve cells, and the presented viral epitopes confront each other it is clear that at equal affinity level, all memory clones will be stimulated and many of them will enjoy a fast start, in case several members of the same clone are able to bind the epitopes. Instead, few of the 10 naïve cells will bind. The condition of equal average affinity for memory clones and naïve cells is found at $D = 1$ or 2. Instead, at $D = 0$ the average affinity of memory clones is overwhelmingly high, and no naïve cell is expected to make it. Only at $D = 3, 4$, and 5, the chances of the memory cells, relying mostly on their number are progressively vanishing. Several of the best naïve cells will get to the Ag. At even higher distances, only naïve cells will have the chance to be stimulated; however, their initial numbers are low (10, if we continue our example) and, therefore, the build-up of the critical mass will take much more time than for the memory, and the peak will be reached only after 180 t_s .

The early bird

The stimulation and start of proliferation of the memory cells opens the second phase of the Tk action, the finding of the infected EC, the binding of the epitope presented by Class I receptor, the killing of the cell, and of all already formed virus particles it contains. This operation is most efficient when the frequency of the effectors is high, and this is the case of memory cells, which were many already at the beginning of proliferation.

Reaching the critical number early in the infection has a double effect, it allows easy eradication of the infection since the viral population is still small, and it creates an Ag draft that pre-empts the stimulation of emerging naïve cells released from the thymus: the early bird has eaten the worm; no more food is available for the late sleeper. And the metaphor continues: if our worm, like a virus, has a high-proliferative rate, then the early bird destroys not a single worm, but an entire population of potential worms, and deprives the food of not one, but a population of its late sleeping competitors.

Mors tua vita mea

The above example is perhaps too childish, but it presents an intuitive explanation of the dramatic suppression of naïve responses (e.g. in the left half of Figures 5 and 6), and has the merit of introducing what is at stake in biology: *survival*, an idea intuitively connected with that of food. The immune response, albeit indirectly, is about the survival of the individual or even, the species. If the bacterium's dream is to become two bacteria [12], the immunocyte wants to live and become a clone. The Th cell rescues the B-cell that has bound and processed its specific Ag by reassuring it about the legitimacy of its action, and by spraying it with lymphokines guarantees the B-clone's survival. The Th cell seems to act automatically, but at the same time has just produced a future for its own clone.

Preservation vs. innovation

Memory is a lurid example of survival of the organism and the species through the preservation of clonotypes that have “worked well once” (never change a winning team). In the same spirit, evolution utilizes the paradigm of cross-reaction and “heterologous response” to preserve the flexibility of the system by upholding the useful and the thwarting of the helpless, in a continuous progress of the quality of defenses. Immunological memory has two assets: the advantage of numbers, and the *suspension of the rules* that other lymphocytes must abide to. In the model, both are incorporated, even if the second should probably be enhanced to be true to life. Of course, favoring memory can have its drawback: in the long run, and the input of new specificities may become wanting.

Our finding through systematic modeling that memory can limit the quality of the response and the size of the naïve repertoire obliges us to pose questions with a practical relevance, and allows us to propose some answers about the mechanisms underpinning somatic evolution of the immune system. One question is: does memory anti-naïve action and actual damage or weakening of the naïves produce capacity to fight invaders? To answer this question we are designing *in machina* and *in vivo* experiments, and preliminary virtual results are already available (see results section).

Strength of today, weakness of tomorrow: Aging

So far, there is no evidence of a decreased efficiency caused by the presence of cross-reacting memory, despite its suboptimal quality. This paradox means that the affinity level is not the only support of efficiency: we have shown here that timing and speed of deployment of responding cells are a fundamental characteristic of the efficient response: even general Ludendorff would agree. If rapid suboptimal memory response does its job, and thus avoids the Darwinian negative selection, the danger of overpowered memory could reveal itself later, even much later.

This could be one of the causes of the aging of the immune system. It seems logical to propose that among the many traits discovered and thoroughly studied, the increased rigidity of the memory hierarchies and the progressive decrease of new responses from naïve cells are at the basis of the old generation of the immune system in aging, and that the MaN tendency in heterologous responses, together with the dwindling of new bone marrow production of lymphocytes are an important part of its mechanism.

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Figure 1.

The success of the challenging virus increases with antigenic distance between primary and challenge. After the challenge by infectants of increasing antigenic distance from the “priming” infection, the success (peak $\times$ life time) is smallest at $D=0$, and increases to the level found in the infection of a naïve virtual mouse, when the clearing is the effect of a primary response. The pattern shows a slow increase up to distance 4, followed by a fast increase from distance 4 to 7.

Figure 2.

Quality and cellularity of the secondary response are affected by the antigenic distance between challenge and priming resulting in biphasic curves. Figure 2a,b shares the same abscissa (antigenic distance between primary and challenge virus); on the ordinate, Figure 2a shows the affinity level (1 = 1, 2 = 7, 3 = 35, and 4 = 35) multiplied by the peak number of Tk cells in each slot; Figure 2b shows the weighted strength, i.e. the same value of the previous figure, divided by the total number of cells in each response: this yields the average affinity of each cell of the response.

Figure 3.
Biphasic change of Tc affinity distribution depends on antigenic distance.

Figure 4.

Initial number of memory cells and the quality of their response depends on the antigenic distance between challenge and priming. Figure 4a shows the level of memory cells present at time 0, when the challenge virus is delivered for each D , the memory cells were elicited by stimulation with a different virus; their number is comparable, about 230–260/virtual mouse. Figure 4b shows the breakdown of the memory cells at each distance $D = 0$ to $D = 7$, according to the level of affinity they have to the challenging virus (legend: color codes of affinity levels). Figure 4c shows the weighted affinity of the same memory cells toward the challenge virus which is the virus that primed the VM in $D = 0$: it is highest for $D = 0$ and goes to 0 at $D = 5$ and further.

Figure 5.

Total Tc secondary response and affinity breakdown as affected by antigenic distance.

Figure 5a shows the cellularity of all Tc (memory or naïve) at the peak of their response to the second virus. Figure 5b is the breakdown of the data by affinity level, (legend: color codes of affinity levels), and Figure 5c shows the strength (affinity $\times$ cell number) of the response.

Figure 6.

Naïve cell response and affinity breakdown as affected by antigenic distance between challenge and priming. Figure 6a shows the number of Tc naïve cells at the peak of their response to the challenge virus. Figure 6b shows their breakdown by activity level, (legend: color codes of affinity levels), and Figure 6c shows the weighted affinity.

Figure 7.

Virus kinetics after challenge, as affected by antigenic distance. Monitoring of virus multiplicity during the second infection in each of the $D=0$ to $D=7$ responses ($T=0$ to $T=300$), Figure 7a,b shows the same data but the Y axes in Figure 7b uses even scales in order to show the real proportions peaks.

Figure 8.

Comparison of the kinetics of viral growth and Tk cell growth. Ordinate: time from challenge to peak. Abscissa: antigenic distance between priming and challenging virus. The peak of the response is at $t = 140$ which means that its maximal virus—killing efficacy is exerted between $t = 100$ and 140 , after which it declines because of the decline of antigenic availability. At higher antigenic distances ($D = 2$ to $D = 7$), the virus reaches its peak at about $t = 140$, because it is not hindered by the response, which is evidently less efficient. Thus, the time of maximal efficiency is faster, only 25 ts separate the peak of virus from the peak of cells, (legend: color codes of affinity levels).

Figure 9.
Viral success and Tk secondary response as affected by antigenic distance.

Figure 10.

Increasing speed of viral proliferation emphasizes life-saving effect of memory when antigenic distance is low and medium. Systematic study of cross-reaction by challenging primed VM with viruses of progressive antigenic distance, and of different duplication speed. (a) Ordinate survival rate. Abscissa: antigenic distance between pruning and challenging virus, (legend: color codes for virus replication speeds) and (b) ordinate survival rate. Abscissa: speed of duplication of challenging virus, (legend: color codes for antigenic distance).

Figure 11.

The abscissa represents mortality and time of death as affected by virus speed. The ordinate shows antigenic distance. In each square (group), the white area is proportional to the fraction of surviving VM. The shaded (area) represents the fraction of dead VM (bubble or full square when mortality is 100%). The intensity of gray increases with increasing speed of death, as defined by the average time of death after the challenge, (see legends on the right).

Scheme 1.

The infection triggers the defense: cellular and humoral. Schematic diagram of the IMMSIM₃ model. At the top middle of the figure, a virus (denoted by a small circle) enters an EC. It grows there, and its peptides are displayed on the EC's MHC1 until it reaches its lethal load, at which point the cell bursts, releasing more viruses and casting a damage signal due to the dead cell components (bottom middle). The damage signal activates an A-cell that has internalized the virus and that can then bind to Th₂ cells (left side of figure), activating them so as to be able to stimulate a B-cell that has bound free virus. The stimulated B-cell proliferates and differentiates into B memory cells and plasma cells, which in turn produce Abs instrumental to the elimination of free virus (top, just left of middle). On the other side, an A-cell that displays viral peptides both on MHC1 and MHC2 can stimulate a Tc cell if it also interacts with a Th₁ cell (right side). The Tc becomes an effector cell that can then kill any EC presenting viral peptides on its MHC1, thereby destroying the load of immature viral particles. Thus, the infectants can be eliminated and the infection resolved by a synergy of Abs binding free virus and effector Tc cells, killing virus-infected cells.

Scheme 2.
Design of the “virus speed” experiment.

Table I

Default parameters of IMMSIM used in the present study.

Parameter	Default values
String length (bit)	16
Mismatches	3
Grid size	15 × 16
Ep death level	0.5
Death load	200,000
B, Tc, Th population	2,500
APC population	1,500
Ep population	20,000
B, Th multiplication	3
Tc multiplication	7
Plasma cell, Ab life time	10
APC, B, Th, Tc life time	50
Ep life time	100
Memory cell life time	20,000
IFN, IL4, damage life time	3
Binding probability APC + Ag	0.001
Binding probability APC + Fc	0.02
Binding probability Ab + Ag at perfect match	1
Mutation parameter	0.2, 1.0, 3.0
Affinity level and enhancer	0.03, 1.1
Ag1 lethality (mult, infect, and lethal load)	1.05, 0.001, 80
Ag2 lethality	1.06, 0.001, 80

Notes: Values different from those listed were also used in order to perform specific experiments.