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A systematic approach to vaccine complexity using an automaton model of the cellular and humoral immune system I. Viral characteristics and polarized responses

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Abstract

A modern approach to vaccination faces the compound complexity of microorganism behavior and immune response triggering and regulation. Since computational modeling can yield useful guidelines for biological experimentation, we have used IMMSIM$_3$, a cellular automaton model for simulating humoral- and cell-mediated responses, to explore a wide range of virus–host relations. Sixty-four virtual viruses were generated by an assortment of speed of growth, infectivity level and lethal load. The outcome of the infections, as influenced by the immune response and the bolstering of cures, obtained by vaccine presensitization are illustrated in this first article. The results of the in machina experiments allow us to relate the success rate of responses to certain combinations of viral parameters and by freezing one or the other branch, and to determine that some viruses are more susceptible to humoral, and others to cellular responses, depending either on single parameters or combinations thereof. This finding allows prediction of which infection may be susceptible to polarized (T helper $(Th)_1 > Th_2$ and $Th_1 < Th_2$) responses and will eventually help designing vaccines whose action relies on antagonizing both the specificity and the behavior of the invader. A second, not lesser, result of this study is the finding that humoral and cellular responses, while cooperating, towards the cure of the infected body, also show significant patterns of competition and mutual thwarting.

Keywords: Vaccination; Computer modeling; Cytopathic viruses; Humoral and cellular responses

1. Introduction

1.1. Vaccines

When the vaccines of Jenner and, later, Pasteur were applied and did work, the concept of vaccination suddenly appeared like a theorem, simple and evident [1,2]. The ideal vaccine seemed to be at hand, sporting all antigenic characters of the eventual invader, minus its danger to the organism. It was up to immunologists to find the way to attenuate the latter without modifying the immunogenicity. More than a century later, this ideal turns out to be both unattainable and far too naive.

There are at least two main reasons why this ideal cannot materialize. The first is that, once physical or chemical forces have been applied and produce 'attenuation', the antigen may not be the same anymore in all its epitopes and peptides, thus presenting an altered antigenicity and/or immunogenicity [3]. Since what counts for immunity to develop is not the absolute capacity to stimulate the immune system, but the congruity with the invader, the vaccine will be weakened in one or both of its missions: building a specific protective response and preparing the memory for a future response, perhaps years later [4]. In addition, both in the case of molecules (e.g. toxins) and of organisms,

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even the alteration of characters that are not directly immunological, such as susceptibility to processing or speed of growth, can ultimately reflect on immunogenicity.

The second reason why the attenuation scheme is problematic became clear with the recent elucidation of a different factor of immunogenicity. It is related to the damage that the antigen (molecule or microorganism) can inflict to the body and it is expressed via inflammatory molecules, eventually providing 'second signals' required for the activation of antigen presenting cells (A cells) and T cells [5,6]. Attenuation, whose aim is removing the capacity to damage, eliminates this important component of immunogenicity.

The entire defense system appears to be far more complex than anticipated. One basic feature of the immune response that increasingly interests vaccinology is that it involves two machineries: the humoral and the cell mediated. There are infections where one or the other are known to be more effective [7,8]; in general, they complement each other, but their life histories are sometimes competitive and most responses tend to be polarized in one or the other direction. Polarization begins with the differentiation of naive T helper (Th) cells into Th_1 or Th_2 early after antigen stimulation [9], even if the hypothesis has been aired that polarization pre-exists in A cells and different A cells activate Th_1 and Th_2 [10]. Only some of the factors (e.g. genetic, environmental) influencing this crucial step have been identified, and it is known that cytokine feedback insures dominance of the initial thrust. However, the final balance of cellular and humoral responses in vivo may not be the most efficient for the victory over the invader, and a 'wrong' balance may not only be less efficacious, but it may also cause immunopathology and autoimmune disease. For these reasons, influencing and re-orienting the balance by the use of specific adjuvants [11–13] is a goal of increasing appeal and importance, and a new engaging assignment for vaccinologists.

Each new problem and each step forward adds to the formidable complexity of vaccines. It is urgent to conduct a systematic study where the impact of a large number of factors (such as viral characteristics including mutability, epitope and peptide distance between vaccine and invader, and polarization Th_1–Th_2 of the response) are evaluated. While this task would be time consuming to perform in vivo or in vitro, and perhaps difficult to justify to biologists engaged in deeply specialized investigations, it is an ideal project for experimenters working on computer models. They can examine large parameter spaces in little time and provide biologists with a large and accurate background for further research.

1.2. Modeling

Most models of the immune system are based on differential equations [14,15]. These models have been used to understand dynamical systems for over 300 years. This experience has led to many formal methods of analysis as well as an intuitive understanding of how many systems behave.

Using differential equations to model the immune system has three main limitations. First, they assume sufficiently large population sizes from which the properties of essentially identical entities can be calculated. However, each cell of the immune system has a unique life history that defines its particular interaction with the environment so that averaging over such a population is often far from adequate. The typical solution to this problem is to divide the cells into a small number of classes based on only a few characteristics. However, this approach ignores the true complexity and myriad of special cases so important to experimental immunologists. The second limitation is that the equations give only the average behavior of a system. Although there are questions for which knowing the distribution of behaviors is not relevant, there are many more questions that cannot be addressed without this knowledge. Finally, the immune system models often involve nonlinearities, which make the solution of differential equations difficult.

The approach that we describe in this paper uses a modified cellular automaton [16,17]. Although our automaton is more complex than the automata usually considered by mathematicians and is not subject to analytical analysis by presently known methods, it has several advantages. For example, since it is stochastic, we can estimate the distribution of behaviors exhibited by the system, not just the average. It also is easy to modify the complexity of the interactions without introducing any new difficulties in solving the model because nonlinearities are not intrinsically difficult to handle in cellular automata. Finally, the automaton is able to represent the components and processes of interest in biological language so that the approximations in the model are more biological in character than mathematical (see, for example, Refs. [16,18]).

To realize this approach, we have used the $IMMSIM_3$ model. An upgraded version of the original model [16,17] include both the humoral and the cellular immune system, and to incorporate the relevant activation steps for the A cells (professional antigen presenting cells) and the Th_1, Th_2 and Tc cells [18]. To represent the challenging invader, we have constructed a spectrum of 64 cytopathic viruses, differing by the assortment of four levels of three basic parameters: speed of growth, lethal load and infectivity. As the vaccine, we have used a dead virus that can be displayed on major histocompatibility complex (MHC) I

and II, but is unable to infect cells or multiply. Since the vaccine does not generate damage and, consequently, A-cell activation signals, it has to be inoculated with adjuvant. The antigenic make-up of the vaccine and of the challenging virus is identical in these first experiments. We have studied the protective capacity of the vaccine versus the 64 viruses, as a function of the dose used in vaccination, and we have determined its relative effect on the cellular and humoral responses separately.

In this article, we describe the functional model of the humoral and the cellular response and the construction of the viruses in Section 2, and the outcome of the experiments of infection by different viruses, and the quantitative and qualitative modification of the response through vaccination in Section 3.

2. Methods and materials

2.1. The model: $IMMSIM_3$

The technique used for the simulations described in this paper is of the same type as used for earlier studies of the humoral system [16,17]. However, the program has been much extended and enhanced to include the cellular branch of the immune system as well as the previously studied humoral branch. These extensions necessitated the inclusion of a number of additional components of the immune system. The present system consists of a space of 240 sites (15×16) representing the body. It contains A cells, B cells, Th cells, Tc cells (1000 of each type) and 20 000 epithelial (Ep) cells. Cells (like molecules), with the exception of Ep, diffuse from site to site. All cells have MHC I receptors; in addition, A and B cells have MHC II receptors, B cells have specific antibody receptors, and all T cells have specific T-cell receptors. All receptors are in the form of eight-bit strings. A new critical parameter has been added to the model called 'Damage', which represents the event that results in activation of the A cells (dendritic cells) and allows the initiation of the immune response. It corresponds to 'danger' in the original concept of Matzinger [5], and has recently been viewed as ''signals released by cells undergoing stress, damage or necrotic death'' [6]. It arises from virus-induced cell death, antigen–antibody complexes, or the injection of adjuvant.

2.2. The grid

As in our previous model, the body section that these components occupy is a cellular automaton grid. The grid is a checkerboard-like arrangement but with each square (site) having six equally spaced neighbors so that the grid is hexagonal in appearance. Furthermore, periodic boundary conditions are used so that the grid has no edges. Entities moving off the grid in one direction appear at the opposite edge. The grid is meant to represent an average piece of the immune system partaking in the immune response, e.g. section of a lymph node.

2.3. The interactions

The significance of the grid is that only entities in the same site can interact on a given time step.

As with all cellular automata, the system is discrete in both space and time. On a given time step, all entities in a given site are allowed to interact and the successful interactions are determined by comparing the probabilities for any interaction to a set of random numbers. The successful interactions are allowed to take place, then a number of other process are allowed to proceed, e.g. birth of new cells from the bone marrow, cell death, antibody production, antigen growth, antigen killing of cells, cytokine production and decay, etc. At the end of all this, the entities are allowed to diffuse to neighboring sites. That is the end of a time step and the process is repeated for as many time steps as desired. Antigen can be introduced from the outside at any time.

2.4. Living antigens

Antigens are composed of one or more eight-bit epitopes (recognizable by B-cell receptors and antibodies) and one or more eight-bit peptides (recognizable by T-cell receptors after processing and in association with MHC). In the present version of $IMMSIM_3$, the concept of antigen was generalized to include live infective antigens (both extracellular and intracellular). In addition to epitopes and peptides, these viral-antigens possess parameters describing their speed of growth, infectivity and lethality. In the experiments in this paper, we simulated cytopathic viruses that do not multiply externally, but begin proliferating once they have infected a cell and continue until the latter bursts, releasing the newly formed infective pathogens.

2.5. The receptors

The cellular receptors and the antigen epitopes and peptides are all represented as bit strings. The length of the string is at the choice of the investigator and is eight for the work reported in this paper. However, we have carried out experiments with up to 16 bits. The diversity of the receptor pool is 2^N, where N is the number of bits. This means a diversity of 256 for the case $N = 8$ and 65 536 for $N = 16$. The interaction between two entities is based on complementary bit strings, i.e. a 'zero' in one string binds to a 'one' in the other. The probability of interaction between entities (B/Ag, B/Th

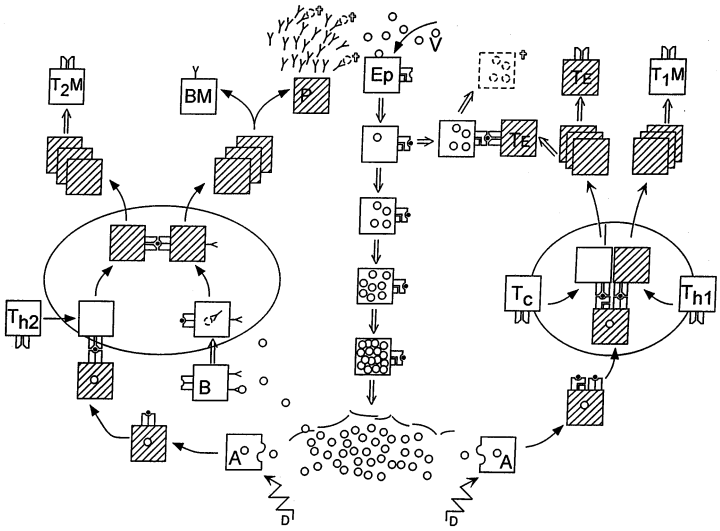

Fig. 1. Schematic diagram of the IMMSIM$_3$ model. At the top middle of the figure, a virus (denoted by a small circle) enters an epithelial cell. It grows there, and its peptides are displayed on the epithelial cells MHC I until it reaches its lethal load, at which point the cell bursts, releasing more 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 Th_2 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 antibodies 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 MHC I and II can stimulate a Tc cell if it also interacts with a Th_1 cell (right side). The Tc becomes an effector cell that can then kill any epithelial cell presenting viral peptides on its MHC I, thereby destroying the load of immature viral particles. Thus, the infectants can be eliminated and the infection resolved by a synergy of antibodies binding free virus and effector Tc cells, killing virus-infected cells.

(through MHC2), Tc/Ep (through MHC I), etc.) depends on the number of matching bits and decreases as the number of matching bits decreases. The details of these interactions as well as the process of presenting antigenic peptides on MHCs are described in Ref. [19].

Only the interactions involving specific receptors proceed as already described. Some interactions, such as A cell/Ag and Ep cell/Ag occur with fixed probabilities. Interactions involving damage have a probability depending on the concentration of damage in the site.

Although the processing of interactions is the same as in the original model [16], the number of possible interactions is much greater. This is due to the increased number of entities in the system, which has more than doubled with the inclusion of the cellular branch of the immune system and the addition of infective antigens. The course of the model immune system under attack by a virus is shown in Fig. 1.

2.6. Dissecting the responses

With these additions, the new IMMSIM$_3$ version can be used to simulate both humoral- and cell-mediated immune response against the same antigen as illustrated in Fig. 1. The two responses can be run contemporaneously, as happens in vivo, but we can also choose to run the responses individually and compare the fate of the same infection when the humoral only response is run, or the cellular only, or when both responses are active.

2.7. Test set of viral antigens

In order to test the performance of this model immune system, we subjected it to a number of viral assaults. The virtual viruses are characterized by three parameters in addition to their specific epitopes and peptides that are identical for all. The parameters are speed of growth, infectivity, and lethal load. We constructed a set of four values for each parameter that covered the range of certain death by viral infection to certain cure by immune system response. The parameter values used for the tests are listed in Table 1. The infectivity is the probability of a virus infecting a cell upon a single contact. The speed of growth is defined as the growth rate of the virus, which proliferates exponentially inside cells. The lethal load, or burst size, is the number of virus particles in an infected cell that cause it to burst, and represents the average size of the new inoculum into the system.

Table 1
Virus characteristics

Infectivity	Speed of growth	Lethal load
0.0001	0.047	10
0.0005	0.088	45
0.0025	0.167	200
0.0125	0.320	900

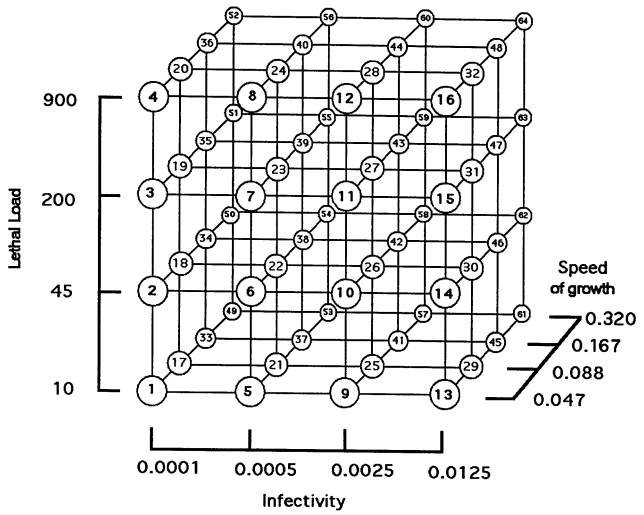

Fig. 2. The spectrum of virus parameters. We have tested the $IMMSIM_3$ immune response against viruses with four different levels of speed of growth, lethal load, and infectivity, and each type is assigned an index from 1 to 64. The parameters are ordered first by lethal load, then by infectivity, and finally by speed of growth, so that the index numbers 1–16 correspond to the slowest replicating virus types and the index numbers 49–64 correspond to the fastest. This diagram illustrates the parameters for each index number.

All combinations were tried, thus the test set includes 64 different viruses; a three-dimensional map relating the number assigned to each virus to the corresponding parameter combination is shown in Fig. 2. All viruses in these tests are cytopathic, i.e. all are able to kill the cell that they have infected.

2.8. Running of infections

Twenty-five runs were carried out for each of the 64 viral types, i.e. 1600 separate runs. For a given type, the only difference in each run was the initial random number chosen. Identical experiments run with different random numbers yield results basically similar but with fluctuations, a condition analogous to running experiments in vivo, repeating the same test in a group of syngenic mice. The initial inoculum is 20 virus particles distributed randomly on the grid. Each run lasts for 2000 time steps unless a resolution (death or cure) occurs before that time. Infections still not resolved are classified as chronic. During the runs, the computer monitors a number of parameters that fully define the ongoing infection and the immune response: (1) the rate of killing of epithelial and antigen-presenting cells; (2) the antibody titer; (3) the free virus; (4) the number of virus particles inside cells; (5) the number and the specificity of activated Tc and (6) of anergized Tc; (7) the number of A cells; (8) the number of infected cells; (9) the number and specificity of Th_1 and (10) Th_2; (11) the number and specificity of resting B cells and (12) of activated B cells; (13) the number of plasma cells; and (14) the number and specificity of resting Th cells. Cure is defined as the complete elimination of virus, while death is determined either by the occurrence of infection of $>50\%$ of the epithelial cells or by a viremia of $>200\ 000$.

2.9. Model of vaccination

We adopt the definition of vaccine as an agent that enhances the potentiality of a response to the challenge by an invader. To measure the extent of this enhancement and to avoid possible noise from the primary response, we delayed the challenge until circulating antibodies from the primary response had disappeared while memory T and B cells still persisted. A vaccination experiment thus consists of injecting a standard dose of vaccine at time -200, followed at time 0 by a standard virus infection. The effect of vaccination is evaluated by comparing the progress of the same infection with and without vaccine. We designed the vaccine as antigenically identical to the infective challenge (same epitope, same peptide); however, we set its infectivity to 0. This makes it a 'dead' virus unable to proliferate since it cannot enter Ep cells. Its in vivo counterpart is the virus-like particle that is used as an epitope carrier in vaccine studies [19,20]. It will not infect cells but it will be internalized by A cells, which will display its peptides on both MHC I and II. However, for this to happen, the A cell must be activated and, since the vaccine is not cytopathic, it will need adjuvant to generate inflammation and a consequent 'damage' or 'danger' signal. As model adjuvant, we used a flat dose of the damage signal, and we determined A-cell activation as a function of adjuvant dose. These results (shown in Fig. 3) led us to choose a dose of 100 adjuvant units to inject along with the vaccine since this activated roughly one-half of the A cells and, while triggering a sufficient immune response, was far from the asymptote.

2.10. Vaccine calibration

Further series of calibration tests were carried out to select the appropriate vaccine doses. Fig. 4a shows that, at low vaccine doses, the time step (TS) of vaccine elimination is large and inconsistent (see large confidence intervals). Fig. 4b,c further show that, at low doses, the immune response is weak (a limited amount of antibodies are produced, and not many Tc cells are activated). Consequently, the ingestion of the free vaccine by the A cells (macrophages) is sometimes the only mechanism to remove it from the body. At higher doses, the antibody response and active Tc response become appreciable and reliable, and the elimination time is short and consistent. Although these results suggest a vaccine dose of 1500 may be yet more effec-

tive, we chose a vaccine dose of 1000 for the vaccination experiments since an intermediate response would be a more sensitive probe to test the differences of the challenging viruses. The dose of both vaccine and adjuvant will be substantially raised in future experiments probing the antigenic distance between vaccine and virus.

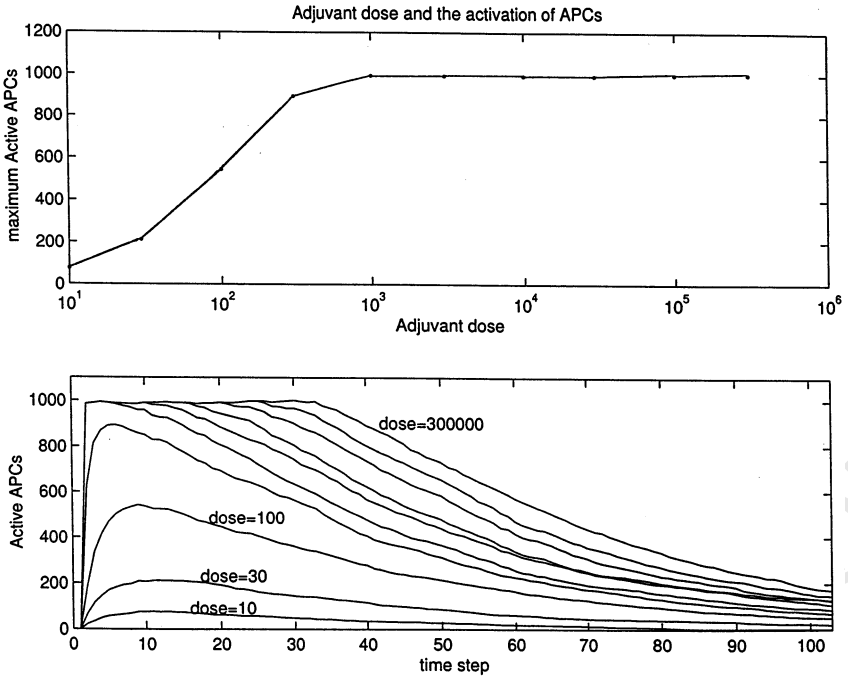

Fig. 3. Professional antigen presenting cell (APC, or A cell) activation by adjuvant. (a) The effect of increasing adjuvant dose (10, 30, 100, 300, 1000, 3000 U) on the maximum number of activated A cells reached throughout the course of a simulation. (b) The activation time course. Saturation is reached with a dose of 1000 adjuvant units, and higher doses only prolong the duration of activation as 'damage' lingers in the system while new A cells are born.

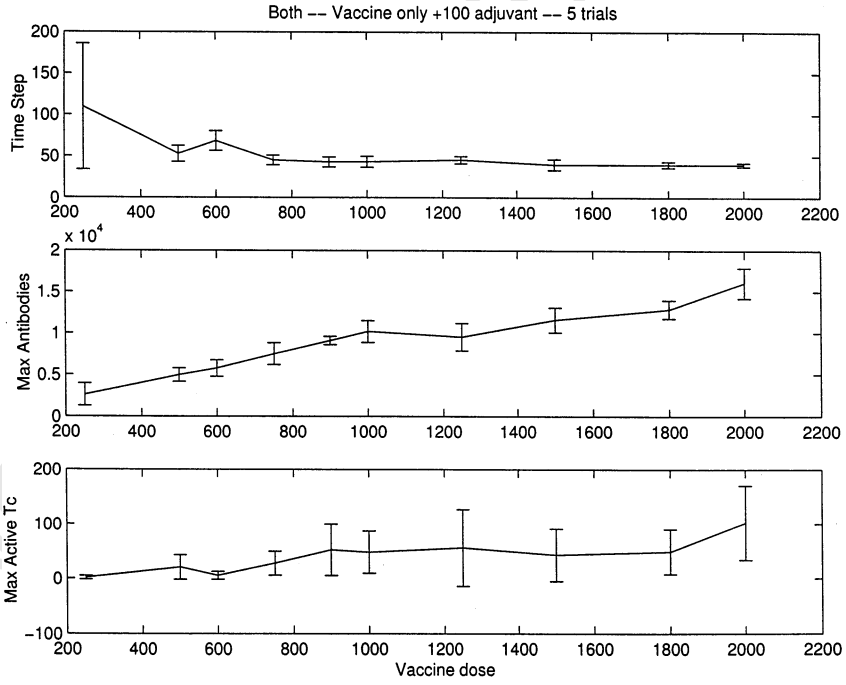

Fig. 4. Immune response to vaccine plus adjuvant. Tests were made with constant adjuvant dose (100) along with a varying dose of vaccine. (a) The number of time steps that elapse before all vaccine is eliminated from the body. (b) The mean maximum antibodies produced. (c) The mean maximum active Tc cells.

Table 2
Overall results of 1600 runs (naive bodies, vaccinated bodies)

	Cures	Chronic cases	Survivors
Both	714, 953	51, 73	765, 1026
Humoral only	252, 309	259, 429	511, 738
Cellular only	34, 134	174, 202	208, 336

A list of the parameters not discussed in this paper can be found at: www.cs.princeton.edu/immsim/papers/vaccine.html. The IMMSIM programs are available for downloading at: www.cs.princeton.edu/immsim/software.html.

3. Results

The 64 viral types were run 25 times on a naive system with different initial random numbers. The entire experiment was repeated three times, allowing both responses or limiting the defense to the humoral only or the cellular only. The simulations were then repeated on vaccinated bodies. The overall results of the 1600 runs in each of these cases are summarized in Table 2. Further breakdown of this data by viral characteristics is shown in Figs. 5–7.

3.1. The response to primary infection

Inoculation of 20 live virus particles in a naive organism led almost invariably to the infection of some epithelial cells, and, after an interval of circa 100 time steps, to the elicitation of both the humoral and the cell-mediated immune responses. At this point, a race typically began between infection and response, which usually ended in either a cure, with elimination of all virus, free or intracellular, or in death of the organism, when virus growth could not be stopped. The divide between the two conclusions was usually reached before 200 TS after infection. Sometimes, it was reached earlier (when either the infection was deficient or the response was incomplete or nonexistent). There were cases where the conclusion took longer to materialize; a few remained neither dead nor cured after as long as 2000 time steps (sometimes with an oscillating behavior) and were considered chronic.

The percent of cases reaching a cure after primary infection with viruses 1–64 is shown in Fig. 5. There are large differences between the three rows, and there are periodic patterns. These are related to the virus index, which is ordered such that speed increases in groups of 16, infectivity has periodicity 16, and lethal load has periodicity 4 (See Fig. 2).

To decipher these results, we consider the impact of the viral quality as well as the impact of the polarization of the response.

3.1.1. The make-up of the virus

The chosen population of viruses is rather aggressive as a group, as the 'complete' immune system secures the survival of less than one-half of the infected. The outcome of each test depends on the combination of characters of the infecting virus. We plotted the survival probabilities as a function of three parameters, speed of growth, infectivity, and lethal load, and represented the results as multiple log contour plots of two parameters at a time (averaging over the third). The three resulting graphs are shown in the top row of Fig. 6. In the left-hand panel, the lines are at approximately $-45°$. Since these are log–log plots, this indicates that the fraction cured depends roughly on the product of infectivity and speed of growth. The two right-hand plots show that the lethal load is less significant since the variation on its axis is much less.

3.1.2. The make-up of the response

The combined humoral and cellular responses are, as expected, more efficient than either one alone, and slightly better than the sum of the two. Considering the results of 25 runs (see Table 2 and Fig. 5, black bars), the overall fraction of survivors for all 64 of the virus types is 48%. The humoral response alone saves 32%, and the cellular response alone 13% of the infected. The advantage of both is more evident when the cures are considered (see Table 2 and Fig. 5): the percent cured is 44.6% for both, 15.7% for humoral only, and 2.1% for cellular only. By comparing the humoral-only response with the both response on single infections, there are many cases where the former is completely useless, but there are definite regions where humoral only is as efficient as the both response. These regions are periodically distributed on the index list. The peaks of humoral only efficacy (numbers 1, 2, 17, 18, 33, 34, 49 and 50) correspond to parameter combinations where the infectivity is lowest and the burst size is low. As far as the cellular-only response is concerned, its efficiency is minimal and occasional cures are found in the regions of infections numbers 2–22. The contour plots can be helpful to better define the issue. Here, the three parameters are compared, two at a time, and their relative weight is deduced by the change in death rate relative to the change of parameter value, e.g. two equally critical parameters will yield a series of contour lines crossing the space at $-45°$. Dominance of one or the other will appear as skewed contours. Let us examine the plots of the second and third row in Fig. 6b (humoral only) and Fig. 6c (cellular only), and compare them with each other and with those of the first row. There is a relative dominance of lethal load and speed of growth in the second row, and of lethal load and infectivity in the third one. This indicates that humoral and cellular responses are functioning best when confronting viruses of different types in terms of their

parameter combinations, and also that the cooperation between cellular and humoral may result in responses with new emerging characteristics.

3.2. Modification of the response by vaccination

The increase in efficiency of the immune response fostered by previous vaccination is summarized by the rise of the overall proportion of survivals after challenge: from 47.8 to 63.9% for both, from 31.9 to 46.1% for humoral only, and from 13 to 21% for cellular only (see Table 2 and Fig. 7). When cures are considered, the corresponding figures are: from 44.6 to 59.5%, from 15.7 to 19.3%, and from 2 to 8.3%.

In both, nine out of ten survivors are cured (without or with vaccine), while this ratio is slightly less than 50% for humoral only (49% without vaccine, 42% with vaccine). In cellular only, the effectiveness is initially very low (16%), but is improved substantially by vaccination (39% of the survivors are cured). The individual data for the 64 viruses and for the three types of responses are found in Fig. 5, where the light bars show

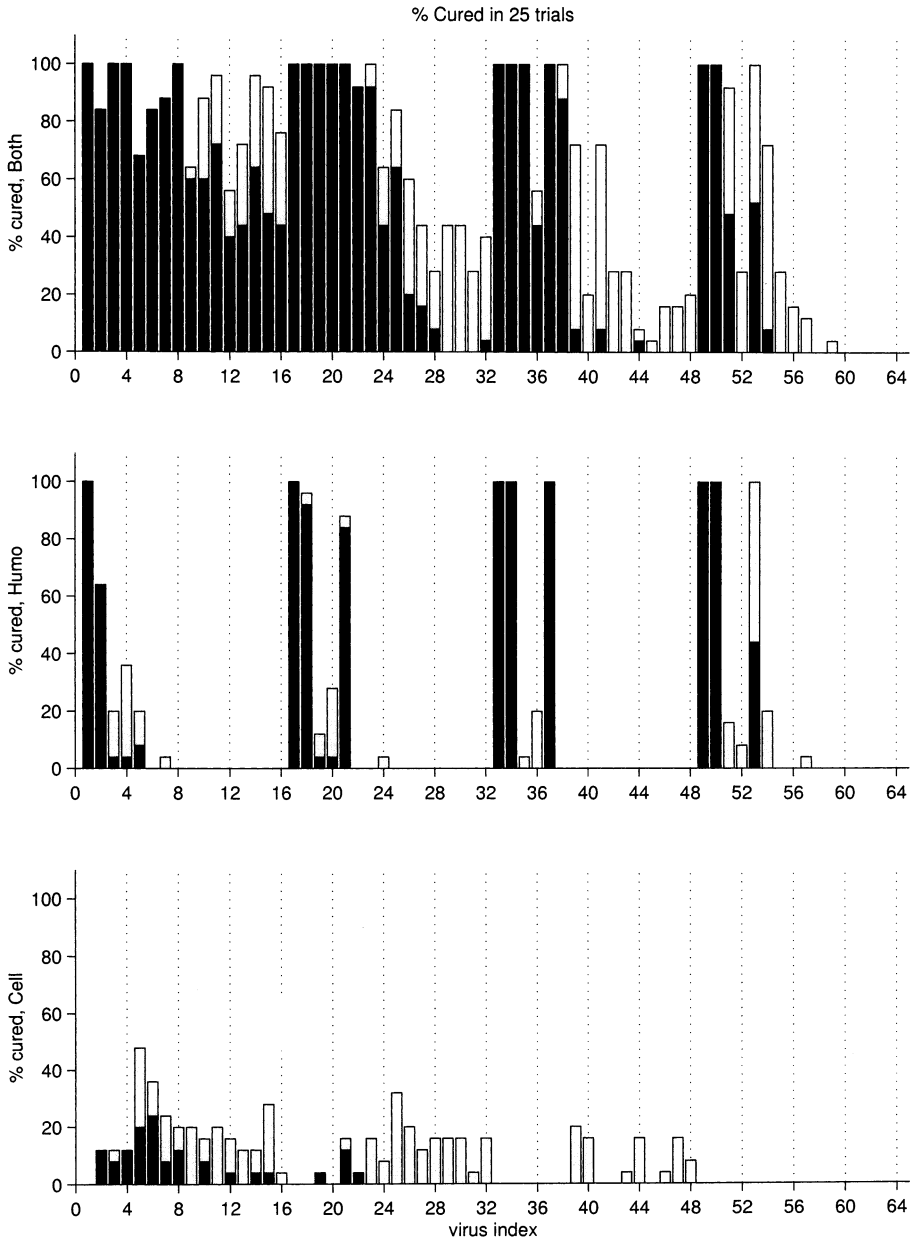

Fig. 5. Cures pre- and post-vaccination. These bar graphs summarize the results of simulations in which the IMMSIM$_3$ model was challenged 25 times by each of the 64 virus types. The x axis has the virus index number as indexed in Fig. 2. The dark bars represent the percentage of cures when viruses are inoculated into naive bodies. (a) The results of both responses, (b) of humoral only, and (c) of cellular only. The lightly shaded bars show the survival percentage increases when hosts are vaccinated.

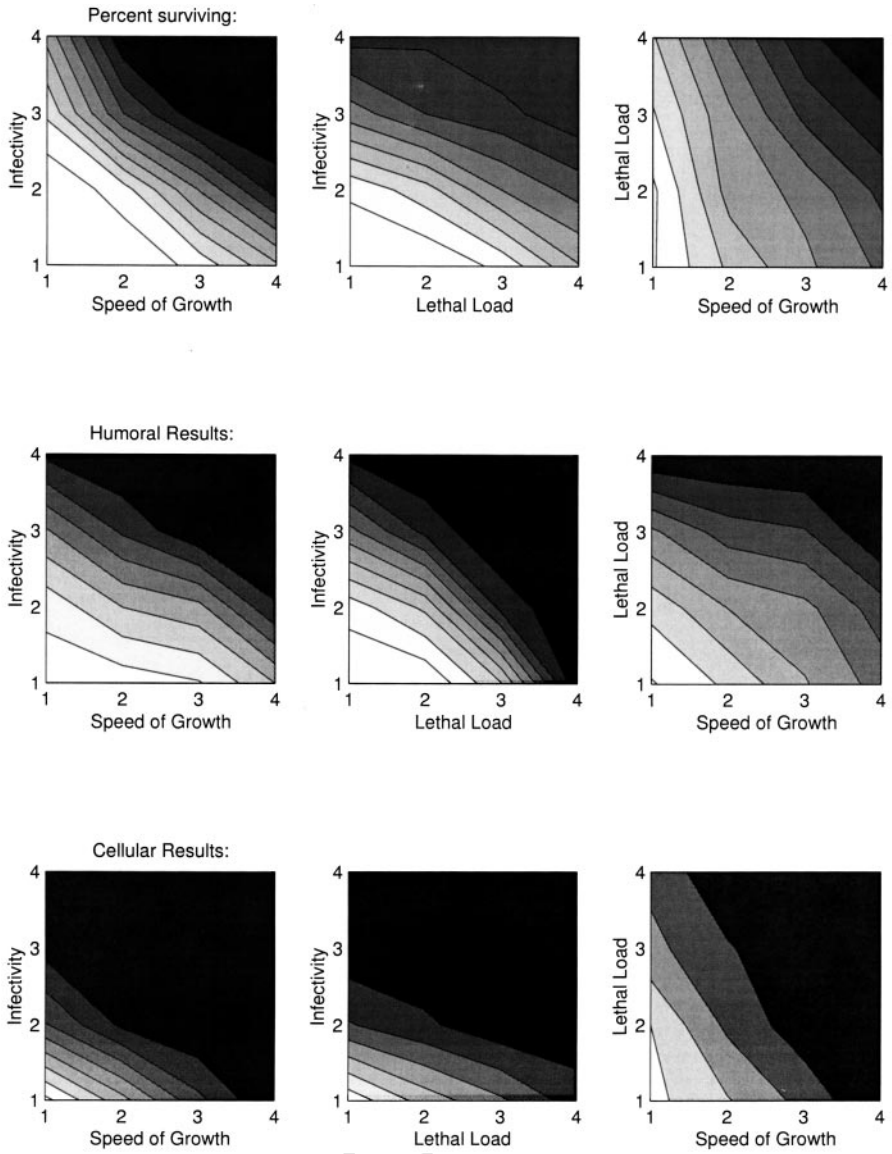

Fig. 6. Contour plots of survival frequency. These contours show survival frequency and the relative impact of two parameters at a time in a two-dimensional plot. Each plot is color coded according to the same scheme: The dark blue area is 100% dead, the deep red area is 100% alive (or 0% dead). Each change of color represents a 10% increment of survival frequency. The three graphs in each row (left to right) show plots of infectivity against speed of growth, lethal load against infectivity, and lethal load against speed of growth. The top row refers to both responses, the middle one to humoral only and the bottom to cellular only. The indications of this figure can be summarized in this way for each row: In row one (both), infectivity and speed of growth are the dominant parameters. In row two (humoral only), speed of growth is less important than infectivity and lethal load. In row three (cellular only), speed of growth and infectivity are the most sensitive parameters.

the increment in the percentage cures caused by vaccination. In both, the improvement by vaccination is evident in all parameter zones where cures were low or absent (numbers 10–16, 24–32, 39–48, and 52–59), except for the last five viruses in the list where vaccination is useless. In humoral only, the improvement by vaccination does not change the patterns of cures, while the opposite happens for cellular only. Here, the cures after vaccine extend considerably towards the center/right section regions of the virus index; although, for each experiment, the percent cures does not exceed 30%, the three shallow peaks resulting fit almost exactly in the regions of the index where the humoral only response is unsuccessful.

3.3. Vaccine and a virulent infection

The series of graphs in Fig. 8 shows the essential events in the course of the infection caused by a virulent virus (number 51), without and with previous vaccination of the host. They display the dynamics of free virus, number of infected cells, antibody production and active Tc response. In the naive body, the

virus infects an increasing number of Ep cells and multiplies rapidly. This causes death before the primary response (active Tc + antibodies) is able to counteract the infection. The injection of vaccine at time −200 causes a primary response (third and fourth row panels). The vaccine is eliminated, and both circulating antibodies and active Tc cells have almost disappeared by time 0 when the live virus is injected. The challenge virus infects cells and begins to multiply, but this time it finds an organism with specific B, Th_1, Th_2, and Tc memory cell clones (not shown). The response, both humoral and cellular, is more rapid and qualitatively and quantitatively stronger; it is able to destroy free virus via antibodies and virus bearing cells via Tc, prompting a complete cure in about 100 time steps.

4. Discussion

4.1. The vehicle

This article illustrates the use of an immune system model based on cellular automata for a systematic study of vaccination. The concept has been extensively tested during the past 7 years by applying it to a number of areas of the immune response such as affinity maturation, hypermutation and rheumatoid factor [21,22]. The present version, $IMMSIM_3$, has evolved to include both the humoral and the cellular response [23,24], and to incorporate recently proposed and demonstrated mechanisms leading to the activation of antigen presenting, Th, Tc and B cells. The first, and appropriate, challenge for the upgraded model is the

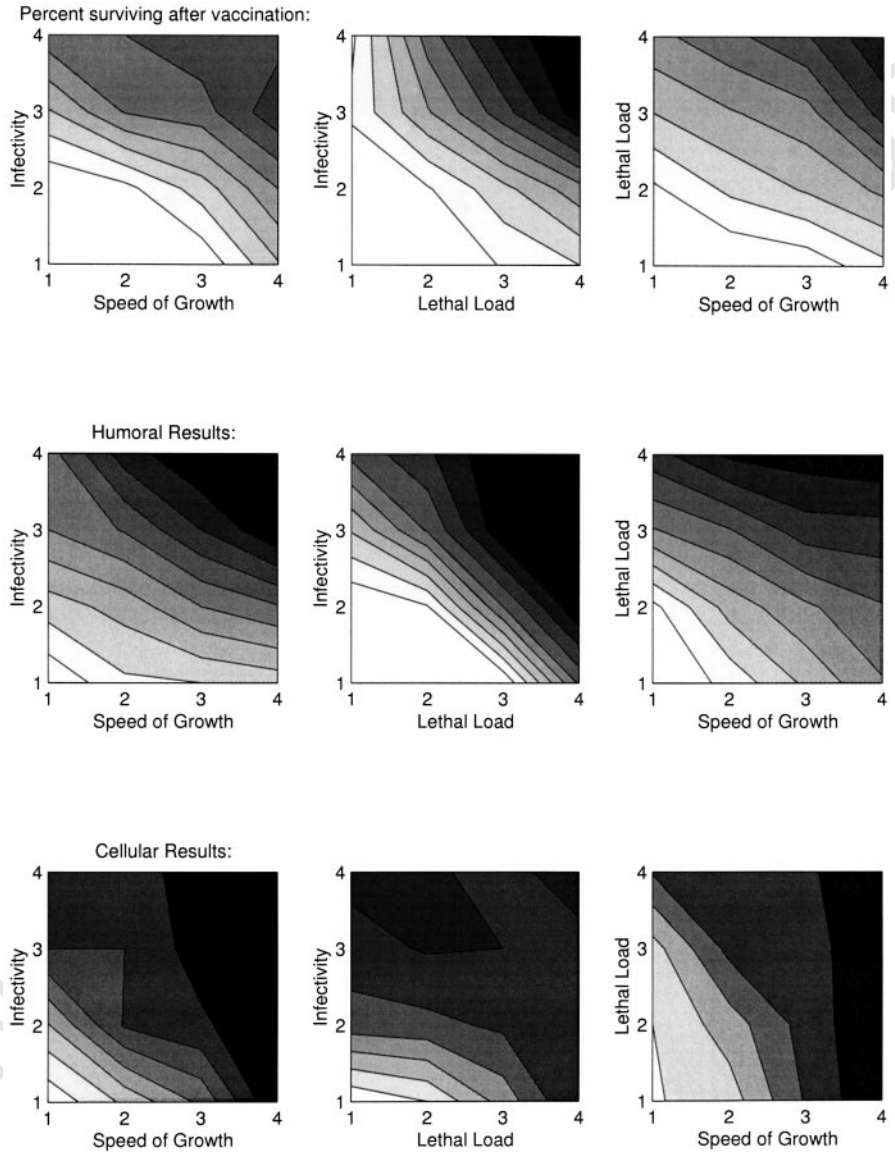

Fig. 7. Contour plots of survival frequency, after vaccination. The data document the change in the quality of the response induced by vaccination. The arrangement of the graphs is identical to Fig. 6. In row one, infectivity and lethal load are the dominating parameters; in row two, infectivity and lethal load again dominate; and in row three, speed of growth is dominating, while the effect of the other two parameters persists.

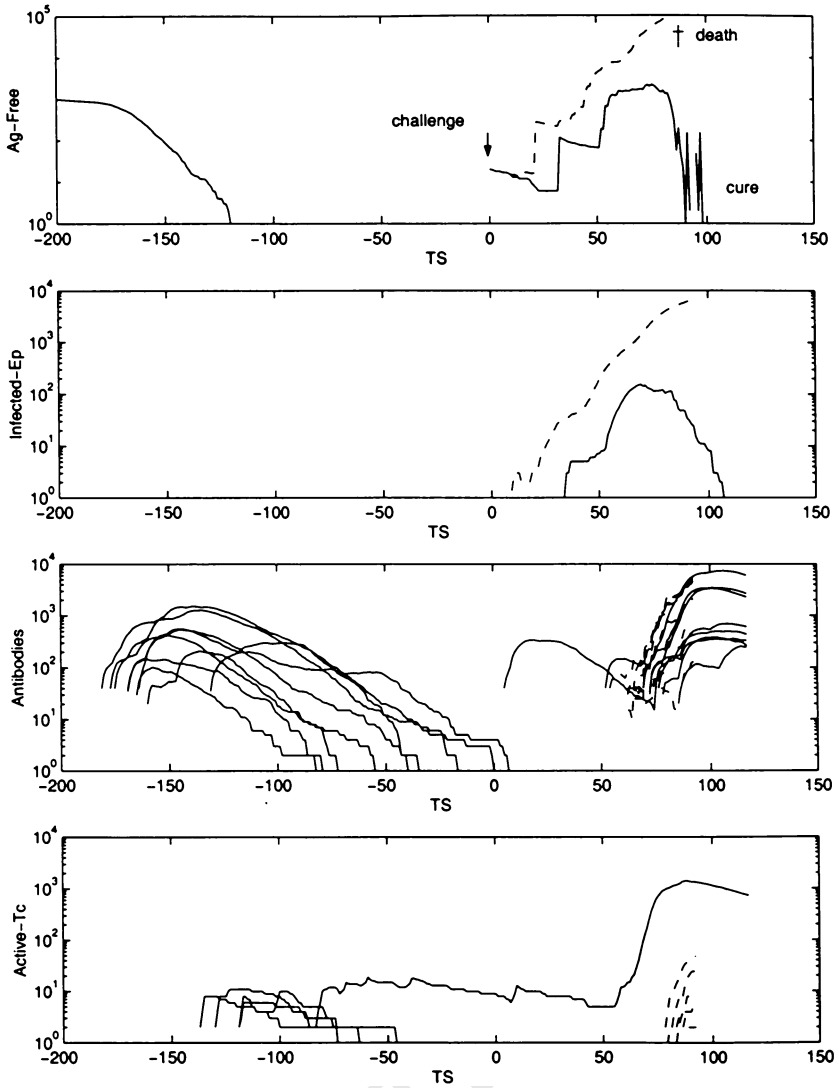

Fig. 8. Infection dynamics. As an example of the impact of vaccination on the course of a viral infection, the response to one of the 64 viruses (index 51) is shown in four rows, without (dotted lines) and with (full lines) previous vaccine priming (at time −200). The following parameters are shown: (1) free antigen/virus; (2) infected Ep cells; (3) antibodies; and (4) active Tc cells. The naive immune system, despite its production of antibodies and Tc, is unable to contrast the rapidly growing infection virus. Instead, the response to vaccine has produced memory cells that react timely and thwart the challenging virus.

confrontation with an infective, cytopathic virus, where both arms of the immune response have a defined role. The humoral response is able to kill or inactivate free virus, and the cellular response is equipped to locate and destroy intracellular sanctuaries of the virus.

Setting up the infectious agent with biological characteristics, and analyzing the essential changes in the immune system and in the body produced by the combined deployment of the infection and of the anti-infection responses, is a significant task per se, and is a necessary preliminary step in the study of anti-virus vaccination. We need to observe: (a) the patterns adopted by the immune responses (humoral, cellular, and their combination) as they are confronted by invaders that display a range of different behaviors; and (b) the specific adaptive moves and countermoves of the immune system when confronted with a range of different immunogenic markers of the viruses.

In vivo, the behavioral and the antigenic diversity of the virus and, consequently, the relative adaptations of the immune system are impossible to extricate from each other, and this adds to the difficulties for experimenters to gain a global view of the infectious phenomenon. Theoreticians, on the other hand, can take advantage of the flexibility of the computational model. We can proceed testing step by step, one variable at a time.

To study vaccination, the model must be endowed with a credible memory and use a realistic vaccine. Immunological memory is the heart of the immune system, but its mechanisms are not completely elucidated [25–27]. While researchers completely agree on

the importance of some invoked mechanisms (increased number of specific B and T cells available, persistence of activated cells), on others, especially those invoked to justify long-lasting memory (persistence of antigen, continuous stimulation, idiotypic network, long-living memory cells), the consensus is far from complete. The simulation of memory of $IMMSIM_3$ is based solely on the increased proportion of specific T and B cells and their selection by antigen, while none of the invoked mechanisms described is incorporated. The resulting secondary humoral response shows realistic onset, strength and affinity maturation patterns, and increased speed of antigen decay [16]. An essentially similar behavior occurs for the cellular branch. Both memories have durations determined by the half-lives of the component cells, B, Th and Tc cells. This is adequate for experiments where, as in the present study, the secondary challenge is delivered at times that are not extremely long compared with the half-lives.

4.2. The findings

In this paper, we present the first part of this work, the section where all viruses used, although widely diverse in comportment, have the same antigenic make-up and the same processible peptides, and where there is a perfect antigenic match between the vaccine and all viruses used as challenge. At this stage, there is some data emerging that we wish to discuss. Their statistics are sound, as they are based on a total of 9600 separate experiments (3×1600 for the infection and 3×1600 for the vaccination, including 25 runs of each parameter set). Some provisional conclusions can be drawn, some predictions can be launched, and some interesting questions raised.

When analyzing the infection of naive hosts equipped with both humoral and cellular capabilities, it is informative to look at the cases where the patients succumb as well as the cases where they are cured. One can either look at the contour plots of Fig. 6a or at the list ordered by the virus' number of Fig. 5. Here, a large proportion of deaths is found in the section with high numbers (numbers 55–64). As can be evinced from Fig. 2, all these numbers label viruses with the highest growth speed. Viruses indexed 55, 60 and 64 have also the highest lethal load of virus particles when an infected cell bursts; while numbers 61–64 display the highest degree of infectivity. This analysis shows that the values assigned to the three parameters are well balanced, since, for example, the highest speed is a formidable weapon for the virus but, taken alone, it is not sufficient to make the infection lethal (see numbers 49–51), while the combined action of the highest degree of the other two parameters may not be sufficient either (numbers 16, 32, and 48).

The first result is the general improvement of the immune response, and its capacity to cure infection, by vaccination. If one considers the infections that were not systematically cured by the naive immune system, the improvement by vaccine is about 20% overall in cures distributed fairly evenly over the virus index (with the exception of the far-right end). The proportion of increased cures may seem modest at first glance: it is not so if we consider that both the vaccine dose and the amount of adjuvant added have been kept at a suboptimal level in order to allow a more critical and more dissecting analysis of the data. This was achieved by utilizing a feature of the model that is all but denied to wet immunologists, running the same infection while blindfolding, in turn, one or the other branch of the immune response and thus comparing their functions and their success rate when alone or in company with each other.

In fact, the most notable results of the first part of the study of anti-viral defense and the effect of vaccination are: (a) the efficiency of humoral and cellular responses as determined by different combinations of viral characteristics; (b) their complex interaction when working together in both responses; and (c) the rather modest capacity to reach a cure in cellular-only responses, attributable to their impossibility to dispose of free virus.

Some of these results are logical and expected while others are emergent findings and, as such, their credibility and significance will have to be verified in vitro or in vivo. Taken together, they contain suggestions that could be extrapolated to biology, and perhaps used as guidelines in the construction of tailored vaccines towards infections. For these reasons, they deserve to be discussed thoroughly.

1. The efficiency of each response in curing viruses that exhibit definite parameter combinations is evidenced by the distribution of cures along the virus type index (e.g. Fig. 5) where certain parameter values recur periodically. This is true for humoral response and for cellular response after vaccination; however, the viral characteristics that correspond to success of the response are different for the two. Humoral response conquers viruses with lowest infectivity and low burst (numbers 1–3, 17, 18, 33, 34, 49, and 50) irrespective of the replicating speed, while cellular response gets rid of invaders with slow to intermediate growth speed and high infectivity (numbers 12–15, 25–28, and 42–48). These results agree precisely with biological observation that rapidly replicating viruses can be efficiently cleared by antibodies (Sindbis, Vesicular Stomatitis) while more slowly replicating viruses are cleared by T cells (e.g. Lymphocytic Choriomeningitis, Vaccinia). The difference of preferred targets is also documented by the patterns in the contour plots (Figs. 6 and 7) but

is dramatically evident in the complementarity of the bar graphs of Fig. 5, corresponding to humoral and cellular, after vaccination.

2. The functional synergy of humoral and cellular responses, predictable from the complementarity just mentioned, is confirmed by the total cures figures of Table 2 and by a slightly more homogeneous pattern of the cures in both bar graphs of Fig. 5. This result is straightforward since, by running together, each response can help overcome a limitation of the other: the helplessness of the humoral in reaching viruses hiding in cell sanctuaries, and the inability of the cellular at killing free invaders.

It was an unexpected observation that, in about one-third of the cases, the humoral-only response, after vaccination, produced more antibodies, and killed more viruses by antibody action than it did in the presence of the cellular response, in the both results. In a similar percent of the cases, more Tc effectors were produced and more infected cells were killed by cytotoxicity in the cellular-only response than in the both response. To illustrate this suppression of effector elements in both, we have plotted the difference of Ab in humoral–Ab in both, and Tc in cellular–Tc in both against the 64 virus types (Fig. 9). The phenomenon is not random and appears to be governed by definite rules, in relation to the make-up of the viruses. The antibody response is higher in humoral only than in both in cases distributed in the left half of the graph, a region where the growth speed of the virus is lowest or low, in a series of narrow peaks rather regularly separated. Only a handful of cases show a better antibody titer in both than in humoral only. Tc effector production is also better in cellular only than in both in one-third of the experiments, and this happens when viral infectivity is lowest (numbers 1–4, 17–20, 33–36, and 49–53). We can ask: when the single response is better, is it also successful in curing the infection or, on the contrary, are many antibodies and many cellular effectors are produced just in those cases where the response is not an easy winner, and the infection goes on for a long time? When answering this question, another distinct feature emerges: the cases of humoral only being better than both almost invariably correlate with clinical success of the single response. However, where cellular is better than both there is a correlation with clinical success only for the cases with low infectivity, while the contrary is true for all cases with high growth speed where, despite a strong Tc response in cellular only, all infections bring the patient to death.

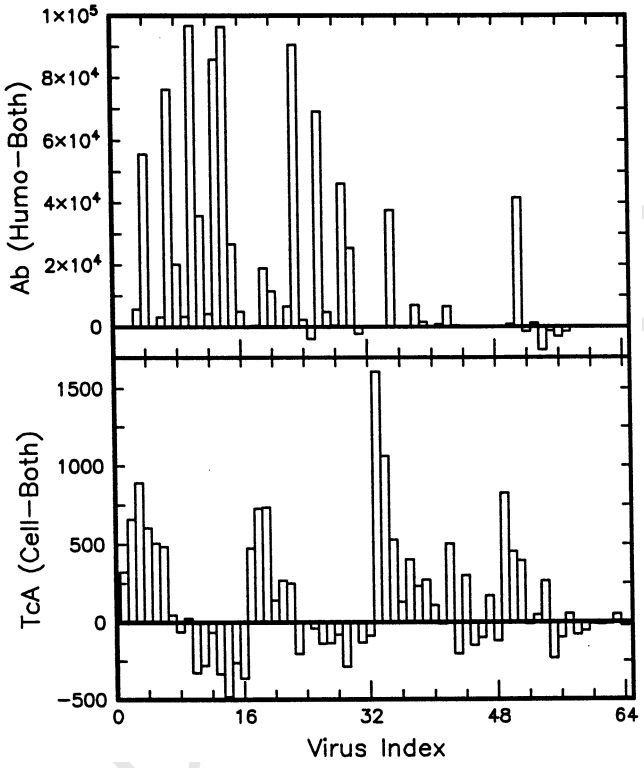

Fig. 9. Effector antibody and Tc-cell differences in both responses as a function of the 64 virus types. Top, The average number (averaged over the 25 runs) of antibodies in both minus the average number in humoral only. Bottom, The average number of activated Tc cells in both minus the average number in cellular only.

In conclusion, it seems that the relations between branches of the immune system are more complex than expected. Some of the results may be explained by competition: for antigen, for Th help, or for the limited number of antigen presenting cells that are capable of activating them. Activation and growth as desirable goals, and the ruthless competition to secure them, remind us of the evolution of species and of cell populations; if food (or antigen) is the cause of the primary competition, then two species of predators (or two branches of the immune system) may credibly try to respond to pressure by enacting additional and more direct means to thwart the competitor. In the present case, the model is not even trying to explain the puzzling Th_1–Th_2 cross-suppression by cytokines observed in vitro and in vivo, but may have indicated a motive.

4.3. Aims for the future

The characters chosen for the virtual viruses and their consequent life styles favor an obligatory cooperation between the two arms of the immune system in order to build an efficient defense. $IMMSIM_3$ has the capability to control the relative strength of the humoral versus cellular response, and the simple device we chose to do it is to either eliminate one cell type (Th_1 versus Th_2) or to act on the ratio Th_1/Th_2, having strictly assigned, to the first, the activation of cellular responses and, to the second, the activation of the humoral branch. We are aware that, in biology, this division of labor is not as strict (Th_1 can stimulate some B cells); in any case, in the present experiment, the

Th_1/Th_2 ratio was kept at 1. However, there are indications (e.g. from the distribution of cures in Fig. 5) that, against some of the viruses, a ratio different from 1:1 would be desirable. This situation has similarities with certain human pathologies (e.g. leprae, AIDS, allergies) where an unbalanced polarization of the response is part of the disease and, consequently, a human intervention aimed at modifying the Th_1/Th_2 balance becomes an engaging goal for immunologists and vaccinologists. The role of the modeler in this context is to provide them with the profiles, in terms of the pathogen's and the host's parameters, of those infections that could profit from a polarizing + immunogenic vaccine versus a simple immunogenic one.

In terms of immunogenicity, there are many compelling tasks, a number of which are presently being carried out and will constitute the matter of future contributions to this work. The first step is to explore the impact of incongruity between virus and vaccine, by conducting experiments in which we alter, bit by bit, the epitope or the carrier peptide of the vaccine, thus moving it antigenically and immunogenically away from the challenge virus. We begin with a one-bit change (1 to 0 or 0 to 1), then with two. The same procedure will be followed for the peptide, then changes in the epitope and in the peptide will be combined and, for all these cases, the residual efficiency of the vaccine will be measured, and the qualitative changes of the primary and secondary response evaluated, by running the 64 infections for each single case. This task is realistic, as is the assumption that any intervention aimed at the attenuation of a pathogen unavoidably alters its antigenic make-up.

Furthernore, we shall include in the simulation viruses that are not cytopathic and diffuse by continuous budding from the host cell membrane and those infecting cells other than epithelial, such as lymphoid cells. The next step toward realism will be the introduction of mutability of the virus during the infection, as its paramount escape route from the immune response. It will be interesting to test how this route and the counterplay of the immune system (e.g. by B-cell hypermutation and affinity selection) is affected by the mutation rates, and determine the boundaries compatible with recovery.

Finally, there is the quest of customizing the viral parameters, with the purpose of substituting the 64 varieties with precise properties according to real pathogens, such as influenza, hepatitis, herpes and HIV, some of which may interfere with the host cells' MHC display and Ag presentation. Although modeling these additions may become increasingly complex, we believe it is at our reach, provided we keep the right perspective regarding what a model is to biology.

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