Chapter 12

Analysing Hypersensitivity to Chemotherapy in a Cellular Automata Model of the Immune System

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12.1 Overview

Most chemotherapeutic agents have proven to induce hypersensitivity. All four types of allergic reactions have been reported in literature, but type I, or IgE-mediated (see below) is the most common one [1]. In the clinical practice these complications are usually overcome by means of either suitable premedication with antiallergic agents, or by postponing drug administration. Nevertheless, the risk of a severe anaphylactic reaction is a major concern, severity strongly depending on the drug dose and the dosing interval between successive injections [2].

Toxic side-effects of chemotherapeutic drugs have been shown to crucially depend on the dosing interval. Moreover, using mathematical modelling it has been conjectured that intermittent delivery of cytotoxic cell-cycle phase-specific drugs, at intervals equivalent to the mean cell-cycle time of the susceptible host cell population (denoted *Z-Method*), may minimise harmful toxicity without compromising therapeutic effects on target cells [3]. These conjectures have been proven analytically [4,5], and generalised for a large class of chemotherapy functions ([6,7]; see also [8]). The predictions of the *Z-method* have been verified in experiments in lymphoma bearing mice, treated by repeated pulse delivery of the anti-cancer drug 1-β-D-arabinofuranosyl cytosine (ara-C). In these experiments it has been shown that when the dosing intervals of drug delivery roughly coincide with the characteristic marrow cell-cycle time, animals survive and myelotoxicity is significantly reduced. The optimal spacing of repeated treatments was determined by measurements of the kinetics of cell movement through different cell-cycle phases [9–12].

The above experiments showed that it is feasible to control host toxicity by rational drug scheduling. With this general concept in mind, we are set to explore methods for reducing a patient's hypersensitivity to a drug, by considering variations in the drug schedule. As in the above-mentioned works, here too we make use of the power and efficiency of the mathematical modelling research tool.

There are many reasons why modelling allergies is an intricate task. The first and most important of these is that allergic diseases, whose origins have yet to be fully uncovered [13], arise because of a malfunctioning of the immune system, which is known to be among the most complicated natural systems. Moreover, modelling allergies has to embrace different levels of biological organisation, going from the gene level (allergies are likely to have genetic origins), to the cell level, through the complicated machinery of cell signalling. A comprehensive mathematical description of this complexity at one time is quite a challenging task. Nevertheless, models which do not take into account the gene-level but focus on the dynamics of population of cells and molecules of the immune system, have already been able to pinpoint very interesting features.

The model we employ is a generalisation of the stochastic cellular automata (CA) concept [14], in that the entities and the rules are not too simplified or stylised. Indeed a great level of description is implemented. This model has been developed on the tracks of a well known CA model of the immune system [15] which describes

the *humoral* immune response to antigens in the host organism (a downloadable C-language version of the original model which includes also the *cytotoxic* response is available [16]).

This chapter begins with a brief background on the topic of allergies and, in particular, on type I or *IgE-mediated* hypersensitivity reactions. Section 12.2 recalls some basic facts and terms related to allergies and to mathematical models of the immune system. Section 12.3 describes the CA model employed, and the necessary approximations made. The results of the simulations are presented in section 12.4. Finally, in section 12.5 we discuss some implications of our results for the design of chemotherapy administration strategies, which may minimise hypersensitivity to the drug.

12.2 Background

The term *atopic* refers to people suffering certain form of allergy. For reasons that are not yet understood, these people have a predisposition to respond to some environmental antigens (e.g., pollen, mold spores) by producing antibodies of the IgE class (immunoglobulin of class E). Since this trait tends to run in families, it probably has a genetic component. It is estimated that over 30% of the world population is atopic. Moreover, the number of people suffering from atopic diseases is increasing in the industrialised countries, revealing a link between modern life and atopy [2].

In the case of allergy induced during the administration of anti-cancer drugs, the patient develops the same type of hypersensitivity to one or more components of the anti-cancer agents, usually during the first or the second cycle of chemotherapy. Among the known cytotoxic anti-cancer drugs inducing an IgE-mediated reactions we recall *cyclophosphamide* [17], *peplomycin* [18], *hyaluronidase* [19], and *paclitaxel* [20].
Although the literature about such cases of hypersensitivity is quite extensive, few articles discuss the possible causes for this immune reaction. Instead, literature usually focuses on elaborating methods for overcoming the problem by means of temporary treatment interruption, administration of antiallergic substances before and during the therapy, or on the use of desensitisation protocols to attenuate the response and increase the probability of tolerating the drug (see reference [21] and cited).

In recent decades, scientists, clinicians, and epidemiologists have elucidated the intracellular and the cellular mechanisms involved in allergic reactions, including the roles of T helper subsets and interleukins [24]. However, our understanding is still lacking as to the full sequence of events involved in disease development, and to the key factors determining the differences between a person who is allergic to, say, grass pollen and one who is allergic to bee venom. The only agreement seems to be that allergenicity is a consequence of a complex series of interactions involving not only the allergen, but also the dose, the sensitising route, sometimes an adjuvant, and

most importantly, the genetic constitution of the recipient [2,13,22].

Many pathologies have been identified as belonging to the class of allergic reactions. In order to further classify them, Peter Gell and Richard Coombs [23] proposed a classification method which is based on the effector molecules and cells involved in the allergic response [24]. The method distinguishes four classes of allergic disorders:

- Type I, *immediate type* or *IgE-mediated*
- Type II, *antibody mediated*
- Type III, *immune complex mediated*
- Type IV, *delayed type* or *T-helper cell-mediated*

Acute or immediate type I hypersensitivity reaction is a consequence of mediators (histamine, leukotrienes, prostaglandin, etc.) released by mast cells (MC) or basophils triggered via the allergen-mediated cross-linking of cell surface bond immunoglobulin-E (IgE). Convincing evidence has accumulated, suggesting that the immune response to allergens in atopics is biased towards the T helper type 2 (Th2) phenotype, characterised by the production of the interleukin-4 (IL-4) and interleukin-5 (IL-5). These are key cytokines in class switching to IgE (replacing IgG) in B cells and in the accumulation and activation of eosinophils respectively [24]. Allergic IgE responses occur mainly on mucous membrane surfaces in response to *allergens* (i.e., common environmental antigen), which enter the body either by inhalation or ingestion. Typically, such responses manifest themselves in localized symptoms, as hives, eczema, hay fever, food allergies, asthma, and systemic anaphylaxis. Most allergens are small proteins, or protein-bound substances, having a low molecular weight. Common antigens, associated with type I hypersensitive reactions are proteins, such as foreign sera or vaccines, drugs, such as penicillin or sulphonamides, local anaesthetics (most drugs are low molecular weight compounds that are incapable of inducing immune responses, unless conjugated with a larger molecule; these small molecules first react with proteins which work as hapten-carriers to form drug-proteins derivatives) etc., (see [24]).

12.2.1 Immunoglobulins and the Isotype Switch

During the primary response of a normal individual, B cells produce antibodies of the IgM type. Several hours after the onset of IgM production, stimulated by the presence of interferon-γ (IFN-γ), IgG-producing B cells swing into action. Eventually, blood serum concentration of IgG antibodies increases above that of IgM, but as long as the antigen is present in the body, both IgM and IgG antibodies continue to be produced. Upon complete antigen removal, B cell stimulation is shut off and the remaining antibodies are catabolised and broken down. Should the same pathogen with the same antigens attempt to reinvade the body, it will stimulate a faster and

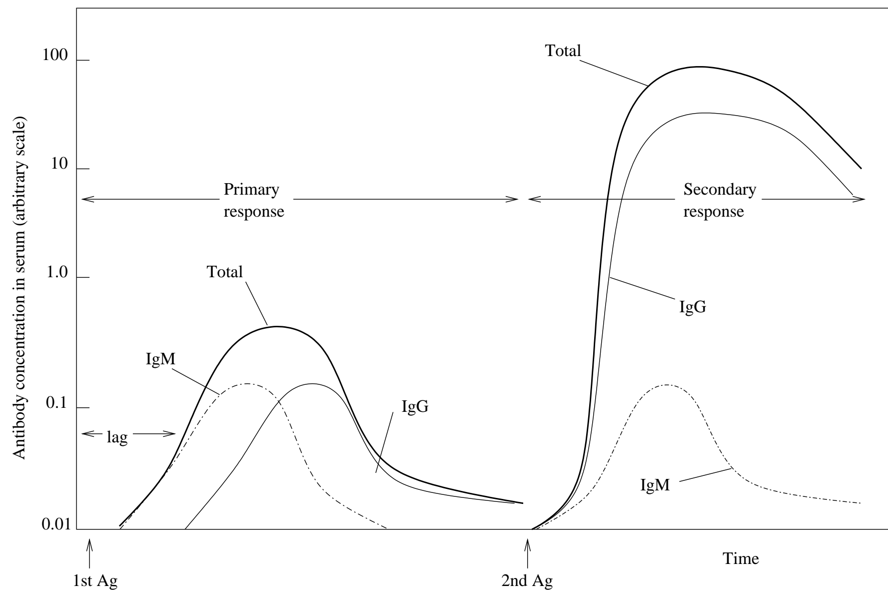

Figure 12.1

Isotype switch during primary and secondary immune response. The concentrations are plotted on a logarithmic scale. The time units are not specified because the kinetics differ somewhat with type of antigen, administration route, species, or strain of animal (adapted from [24]).

stronger antibody production (secondary response in Figure 12.1). This time the IgG antibody producing cells proliferate and release IgG just as quickly as the IgM producing cells. The above pattern of the immune reaction in a normal individual is altered in hypersensitive subjects, mainly by IgE antibodies being produced instead of IgG antibodies. This *isotype switch* takes place in stimulated B cells in the presence of certain cytokines produced by T helper cells [24]. A "normal" isotype switch to IgG occurs if the concentration of interleukin-12 (IL-12) is relatively high, whereas a switch to IgE is dependent on the concentration of IL-4. The problem in having high levels of IgE serum is that they bind to mast cells and basophils through the Fc receptor on the cell membrane, thus *sensitising* these cells. A subsequent exposure to the same allergen induces cross-linking of IgE-bound molecules on sensitised cells. Cross-linking is a term indicating a complex series of events which signal a cell to *degranulate* and release active mediators, such as histamine, serotonin, proteases, eosinophil chemotactic factor (ECF-A), neutrophil chemotactic factor (NCF-A), platelet-activating factor, leukotrienes, prostaglandinis, etc. Finally, the presence of these active molecules provoke a sequence of events, culminating in the symptoms of hypersensitivity. For example, the leukotrienes mediate broncho-constriction, increased vascular permeability, and mucus production (as seen in asthmatics) [24,25].

12.2.2 Cytokines Production and the Role of Th1/Th2 Shift

T helper lymphocytes are mainly classified according to the types of cytokines they secrete [26]. Two distinct kinds of T helper lymphocytes can be distinguished, namely Th1 and Th2 lymphocytes. Th1 lymphocytes participate in cell-mediated immunity. They secrete interleukin-2 (IL-2), IFN-γ, and TNF to enhance inflammation and antiviral responses, and are essential for controlling such intracellular pathogens as *listeria* and *mycobacterium tuberculosis* (the bacillus that causes tuberculosis). In contrast, Th2 lymphocytes provide help to B cells and, in so doing, are essential for antibody-mediated immunity, controlling extracellular pathogens in blood and other body fluids.

Normal immune response requires a balanced activation of Th1 and Th2 lymphocytes. Indeed, many pathologies are related to, or arise from, an imbalance in the activation of these two lymphocyte populations. It has been suggested that the activity of the immune system *in utero*, primed by common environmental allergens crossing the placenta, is very important in determining the individual Th1/Th2 balance and the predisposition to hypersensitivity. This theory states that the immune response of virtually all newborn infants is dominated by type 2 T helper cells and that during subsequent development, the normal infant's immune system shifts in favour of a type 1 T helper cell-mediated response to inhaled allergens. In contrast, in the potentially allergic infant there is a further increase in type 2 T helper cells, which were primed *in utero*. Microbes are probably the chief stimuli of protective type 1 T helper cell immunity [2,22].

A mathematical model of Th1/Th2 balance during adult immune responses has been developed to understand the "decision" of the immune system to trigger a Th1 or Th2 immune response, and how it influences the disease outcome [27]. The model proposes the innate immune recognition as the mechanism for the "decision-making" process. Given this assumption, this model indicates that:

- the default response to pathogens is primarily a Th1 response, followed by a Th1$\rightarrow$Th2 switch, in case of a failure of the Th1 response; and
- antigen dose-dependence of the T helper ratio (high antigen levels promote a Th1$\rightarrow$Th2 switch) and an initial Th1 bias are crucial for the function of selection process.

More simply, in our model, we identify, as a working assumption, the Th2 phenotype as being responsible for hypersensitivity (see paragraph 12.3.1).

Together with Th lymphocytes, the macrophages (MA) are the main source of the different interleukins. Among others, they secrete IL-12 which induces Th differentiation into the Th1 subset. Macrophages are not the only source of IL-12, as any antigen presenting cell (e.g., B cell) is able to secrete IL-12 [24]. The interleukin, IL-12, promotes Th lymphocyte's enhanced secretion of IFN-γ. Conversely,

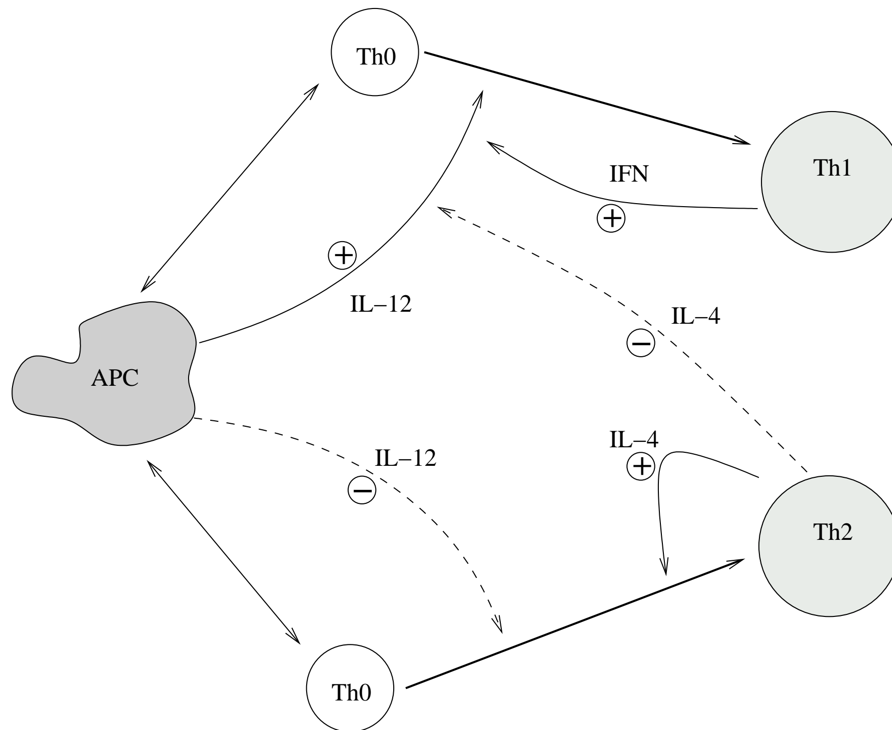

Figure 12.2

Schematic representation of the effects of the cytokines IL-4, IL-12, and IFN-γ and the commitment of Th1 and Th2 cells from the common precursor Th0 in the present model. Dashed lines indicate inhibition, whereas solid lines indicate stimulatory effects.

IFN-γ promotes IL-12 secretion so that there is a positive feedback between these two cytokines (see Figure 12.2). In contrast, by driving the Th response to the Th1 phenotype, IL-12 acts as a suppressive agent of the allergic immune response [28] (observation cited in [29]).

12.2.3 Mathematical Models of the Immune System

The immune system has some unique features, which render it appealing for mathematical modelling:

- It is a highly distributed system, which carries out a complex recognition and classification task

- It evolves and matures using combinatorial, evolutionary and adaptation mechanisms
- It is able to "remember"

Immune system models can generally be classified into continuous models, describing the immune process by sets of differential equations, and discrete models, describing the immune process as a series of interactions in discrete time steps, or utilising combinatorial methods to predict immune properties.

Traditionally, the approach to modelling the immune system involved ODE (ordinary differential equations) or PDE (partial differential equation) [30]. However, in the last two decades discrete mathematical models, and most notably, the CA approach, have become increasingly popular in the theoretical immunology community. These new trends were largely due to the wide-range use of CA in modelling complex phenomena in physics, biology, finance, and, more recently, sociology [31]. Below we briefly overview models belonging to each modelling group. A more detailed review can be found in [30].

12.2.3.1 Continuous Models

Most mathematical models in immunology employ systems of differential equations to describe the dynamic interactions of immune cells and pathogens. The system's description may include equations and parameters for proliferation and death rates of pathogens and lymphocytes, for the transitions between resting and activated states of immune cells, or between naive and memory phenotypes, transitions of the response between humoral and cellular activity, etc. Among the issues addressed using this approach are the maturation of the humoral immune response, exhibited by B cell proliferation and differentiation using clonal selection and somatic hypermutations [32,33], the effect of feedback in monitoring, balancing, and improving the immune response [34], the role of cross-reactive stimulation in maintaining immune memory [35], the threshold ratio between Th memory cells and antigen dose needed to establish T cell memory [36], antiviral immune response in infections, such as hepatitis B, influenza [37,38], HIV [39–42], etc.

12.2.3.2 Discrete Models

One subclass of immune system models uses methods of discrete mathematics to evaluate characteristics of the immune system and to predict its behaviour. Perelson et al. [43] have employed a "shape space" model to study aspects of the immune repertoire: how large should this repertoire be in order to be complete, and what is the probability of recognising foreign vs. self antigens. The shape space model geometrically describes the immunological receptors as points in a multi-dimensional space, each dimension representing a binding parameter such as length, width, charge, etc., and each receptor can bind epitopes within a small "recognition ball" surrounding its complement in the shape space.

A different approach was introduced by Agur et al. [44,45], who analysed the strategy of the humoral immune response as an optimisation problem. Agur et al.

employed dynamic programming methods for investigating the optimal mutation rate function in B cells, which maximises the probability that the required structure of the antigen-binding antibody will be efficiently generated during any immune response. Analytical results have pinpointed a step-function mutation rate as the globally optimal strategy, transition from minimum mutation rate to the maximum biologically possible mutation rate occurring when the size of the best performing B cell clone exceeds a well-defined threshold.

A second subclass of discrete models is that of CA. Discrete in both space and time, these models describe the immune system dynamics by deterministic rules of cells, molecules and their local interactions. In [46] the concept of "evolutionary" experiments *in-machina* (i.e., within computer) was introduced. Thus, computer simulation experiments were performed, where each B cell was represented by a two dimensional cellular automata with variable processing rules. Results of this work suggest that efficient immune response to antigenically homogenous pathogen favours strong contraction in phase space in antibody generation (*one B-cell clone – one antibody*), whereas efficient response to antigenically varying pathogen should favour weak contraction in phase-space in antibody generation (*one B-cell clone – many antibodies*).

These type of experiments can be used prior to any *in vitro* or *in vivo* experiments for qualitatively examining problems in immunology by fast, reproducible, and cheap means. Indeed, Celada and Seiden put forward such a CA simulation model, which attempts to capture "all" the different constituents of the immune system in one comprehensive framework (to be denoted *CS-model*, [15,47,48]). This model has been used to study various phenomena, including the optimal number of human leukocyte antigens (HLA) [48], the autoimmunity and T lymphocytes selection in the thymus [49], antibody selection and hyper-mutation [47], and the dynamics of various lymphocyte populations in the presence of viruses, which are characterised by infectivity, reproduction efficiency, etc., [50]. Formally, the CS-model belongs to a subclass called "stochastic CA."

12.2.3.3 Stochastic Cellular Automata

Most simulators of the immune response are deterministic, assuming that a given set of initial conditions leads to only one end-state. Typically, deterministic models, constituted by a set of differential equations which represent the interactions among immune cells and molecules, are solved iteratively by numerical integration. However, the assumptions underlying the deterministic modelling method cannot represent many intra- and inter-cellular processes, which, typically, are sensitive to the behaviour of a relatively small number of cells and molecules. Under such circumstances any given set of initial conditions can lead to a plurality of end-states. Stochastic CA are models designed to represent the latter systems. In these models the caveats of the deterministic approach are avoided, since they allow for randomness in the activity of the system's operators.

12.3 A Cellular Automata Model of Hypersensitivity

The model to be employed here for studying the role of drug schedules in the eruption of hypersensitivity, is based on the *clonal selection theory* of the Nobel Price laureate F.M. Burnet (1959) developed on the tracks first highlighted by P. Ehrlich at the beginning of the twentieth century.

In our model, a cubic millimetre of blood serum of a vertebrate is mapped onto a two-dimensional $L \times L$ hexagonal lattice (six neighbours), with periodic boundary conditions. Physical proximity is modelled through the concept of lattice-site. All interactions among cells and molecules take place within a lattice-site in a single time step, so that there is no correlation between entities residing at different sites at a fixed time. The diffusion of entities at the end of each time step introduces correlations and is meant to model physical spread of cells and molecules.

Cells are added through an external compartment, which simulate the bone marrow and the thymus. The thymus is implicitly modelled through positive and negative selection of immature thymocytes before they get into the lymphatic system [49]. Major classes of cells of the lymphoid lineage (lymphocytes T helper and cytotoxic, lymphocytes B and deriving antibody-producing plasma cells) and some of the myeloid lineage (macrophages and mast cells) are represented.

The interactions among cells and molecules determine their functional behaviour. They may be *a-specific* (e.g., antigen phagocytosis by monocytes or macrophages, binding by mast cells, etc.) or *specific* according to their affinity or degree of chemical binding strength (e.g., Th interacting with B cells for antigen recognition, etc.). The complete list of interactions is reported in Table 12.1.

In principle, this stochastic CA model allows all cells to interact among themselves. However, in practice, the interactions follow a “greedy” paradigm. That is to say that once two cells successfully interact with each other, they are taken out of the pool of interacting entities for that time step.

Our model is more complex than the majority of the immunological models, as it considers an additional level of description, namely the intracellular processes of antigen digestion and presentation. This *endocytic* pathway is implemented by assuming that the exogenous antigen is digested and attached to the molecules of class II MHC for presentation to the Th’s receptors (further details can be found in previous publications about the original CS-model [15] and its modifications [51]).

At each time step of the simulation of our model all cells and molecules can interact locally (i.e., on each lattice site) according to their internal state, represented by suitable internal variables. An interaction between two cells is considered successful if a change in their internal state has occurred.

The present model differs from the original CS-model, mainly in explicitly representing the cytokines. Among the multitude of cytokines involved in an immune response only a subset will be taken into account in the present model (see Figure 12.2).

Table 12.1 Interactions among cells, or cells and molecules; antigen digestion and presentation on class II MHC by APCs. Other modules of the model. (IC = immunocomplex)

Interactions	Entities involved	MHC class involved
B phagocytosis of antigen	B, Ag	
MA phagocytosis of antigen	MA, Ag	
MA phagocytosis of IC	MA, IC	
APC's presentation to Th	MA, B, Th	class II
Immunoglobulins - Ag interaction	IgE, IgG, IgM, Ag	
Sensitisation of MC	MC, IgE	
Degranulation of MC	MC, Ag	
Digestion and presentation	**Entities involved**	**MHC class involved**
B digestion	B	class II
MA digestion	MA	class II
Other procedures	**Entities involved**	
Desensitise MC	MC	
B's isotype switch	B	
Th's class switch	Th	
Clone division	B,Th	
Haematopoiesis	B,Th,MC,MA	
Plasma secretion of immunoglobulins	PLB	
Diffusion	cells and immunoglobulins	

These are the ones which are directly involved in the allergic reactions [52,53] as follows:

- Interleukin-2 (IL-2), which is secreted by stimulated T helper cells. IL-2 is also known as T-cell growth factor (we will be using the acronym T-GF herein, which is not to be confused with the tumour growth factor). It promotes clonal expansion and differentiation of additional T helper and B cells.

- Interleukin-4 (IL-4), which stimulates antibody-producing B-cells to produce IgE instead of IgG. IL-4 inhibits IL-12 released by macrophages and Th1 proliferation. It promotes Th2 clone expansion instead.

- Interleukin-12 (IL-12), which acts in a contrasting manner to IL-4. It promotes Th1 type response and strongly stimulates T cells to synthesise IFN-γ ([54], observation cited in [29]).

- Interferon-γ (IFN-γ), which is secreted by Th1 cells and induces antibody switch to IgG. It also stimulates IL-12 production ([55], observation cited

in [29]) so that there is a positive feedback between IFN-γ and IL-12 (see Figure 12.2).

It is worth noting that two other cytokines which are often mentioned in the literature to be involved in allergic type I reactions, namely IL-13 and IL-5, are not implemented in the present model, for the following reasons:

- IL-13, which is a IL-4 homologue, is only moderately involved in isotype switch and is not involved in Th2 polarization, although it is significant in other proallergic functions.
- IL-5 is ignored for simplicity, since it is involved in the recruitment and development of eosinophils, which are believed to play a central role during the late-phase allergic reaction [56] but which are not taken into account yet. Indeed, in a further work we will investigate the influence of IL-5 and eosinophils on the problem under study.

Homeostasis is explicitly modelled by a mean-reverting process around the initial population of cells (but see [57] for a simple discrete model of blood cells development, where homeostasis is maintained by simple negative feedback on the phase-transition of each proliferating cell):

$$\Delta X_i(t) = \frac{ln2}{\tau_i}(X_i(0) - X_i(t)) \tag{12.1}$$

where $X_i(t)$ is the number of cells or molecules of class i at time t. Equation (12.1) guarantees that, if no antigen is injected into the system, no interactions take place and the system fluctuates around its initial state. The parameter τ_i indicates the half-life of entity i. Most of these values, reported in Table 12.2, are known from the literature [24]. Exceptions are the half-life of memory cells and plasma cells. It is known that some memory cells live for years or even decades, but it is very difficult to actually estimate their half-life [58]. Plasma cells are believed to live for a few days only, but see [58] for a different estimation. We arbitrarily choose to set the half-life of memory cells to six months and those of plasma cells to three days.

The *paracrine* and *autocrine* nature of the action of cytokines is provided by the fact that cytokines release from cells is *local* and *instantaneous*. That is to say that in our model the cytokines are released at the time a cell receives the required signal (mainly during a receptor-binding with another cell), and they are released locally, on the lattice-site where the interaction takes place.
Here we need to make some working assumptions:

- All cells release either the same *basic* amount (indicated by ω) of cytokines or they secrete an *enhanced* number ($\rho \cdot \omega$) of cytokines (see Table 12.3).
- The enhancement corresponds to doubling the rate of secretion (i.e., $\rho = 2$).
- The basic amount ω is equal for all cytokines. This implies that all cytokines have the same ability of exercising their action.

Table 12.2 Half-life of cells and molecules (τ is expressed in days) [24]. Half-life of memory cells is set to six months although it is believed that the immune memory can last for several years. Half-life of the antigen is arbitrarily set to about a year but, in practice, this value does not play a role in our simulations, where antigen is phagocytised by antigen-processing cells in a much shorter period of time. Histamine lasts for less than a minute but, technically, its half-life is constrained by the time resolution adopted, which is eight hours.

Cell	τ	Molecule	τ	Molecule	τ	Molecule	τ
B	3.3	Ag	365	IgM	5	IFN-γ	0.3
Th	3.3	IC	30	IgE	2.5	IL-4	0.3
MA	3.3			IgG	23	T-GF	0.3
MC	3.3					IL-12	0.3
PLB	3.3					HIS	0.3
B memory	180						
Th memory	180						

The antigen injected into the system breaks the equilibrium, bringing the collective dynamics to a metastable state of infection. In other words, once we inject the antigen, some cells move from the inactive state to the active one, through the interaction with other cells or molecules. A cascade of events follows, leading to the clonal expansion of lymphocytes. For example, during the antigen-recognition process, the lymphocytes T helper interact with the antigen presenting cells and eventually enter the mitotic cycle.
The probability for a stimulated B cell to divide at each time step for a maximum of n steps is given by

$$\mathrm{Pr}[\mathrm{B\,divides}] = F_1\left(T_x(t)\right) \cdot F_2\left(T - GF(t)\right), \tag{12.2}$$

where $T_x(t)$ is the total number of cells

$$T_x(t) = B_x(t) + Th_x(t) + MA_x(t) + PLB_x(t) + MC_x(t)$$

in site x at time t,

$$F_1(T_x(t)) = \exp\left(-\frac{T_x^2(t)}{\gamma^2\left(\sum_x T_x(0)\right)^2}\right). \tag{12.3}$$

γ is a constant which determines a size-effect constraint on the clonal expansion,

$$F_2(T - GF(t)) = 1 - \exp\left(-\frac{T - GF_x^2(t)}{\eta^2}\right) \tag{12.4}$$

is the stimulation given by the local amount of cytokines T-GF, and the parameter η represents its efficiency.

Clone division of cells is governed by several parameters. One of these is the number of duplication steps n (i.e., the number of times a cell creates a copy of itself). This parameters is set to $n = 5$. Hence, under suitable conditions (presence of growth factor or absence of inhibitor cytokines), 2^5 cells are created out of the first progenitor activated cell. The parameter γ in Equation (12.3) is chosen to allow a 4- to 15-fold increase of lymphocyte counts during acute infections [24].

After division a B cell matures into either a memory cell or an antibody-producing plasma cell, with probability 1/2.

The probability for a Th cell that has already entered the mitotic cycle to divide is computed taking into account also cytokines' inhibition.

$$\Pr[\mathrm{Th\,divides}] = F_1(T_x(t)) \cdot F_2(T - GF(t)) \cdot F_3(c), \tag{12.5}$$

where F_1 and F_2 are as in Equations (12.3) and (12.4) respectively, and

$$F_3(c) = \begin{cases} 1 & \text{if } c = 0; \\ \exp(-IL4_x^2(t)/\kappa^2) & \text{if } c = 1; \\ \exp(-IFN_x^2(t)/\kappa^2) & \text{if } c = 2. \end{cases} \tag{12.6}$$

The factor $F_3(c)$ stands for the inhibition of the cytokines IL-4 and IFN-γ, and is dependent on the class $c = 0, 1,$ and 2 of the Th cell: If the duplicating Th cell is of class 1 then it is inhibited by IL-4; on the other hand, if it is a Th2 cell then it is inhibited by IFN-γ. If the cell is Th0 then $F_3 = 1$ and there is no inhibition. The parameter κ represents the cytokines inhibition efficiency (note that, for simplicity, κ has been taken equal for both IL-4 and IFN-γ).

The switch between Th's classes depends on the local concentration of cytokines. The probability p of a Th0 cell to become a Th1 or Th2 is given by the relative local amount of IL-4 and IFN-γ

$$p = \left(1 + \frac{IFN_x(t)}{IL4_x(t)}\right)^{-1}. \tag{12.7}$$

This means that Th0$\rightarrow$Th2 with probability p whereas Th0$\rightarrow$Th1 with probability $1-p$. If neither IL-4 nor IFN-γ is present in site x, then no switch takes place. Note that in so doing, we are actually embracing the hypothesis that it is not possible for Th1 or Th2 committed cells to switch back to the other class. Hence Th1/Th2 are taken as committed cells.

The isotype switch occurring to B cells is modelled through a sigmoid-Hill function with coefficient m and C,

$$p = \frac{x^m}{C^m + x^m}, \qquad C = \omega,\ m = 2. \tag{12.8}$$

The parameter C has been set to ω, which, in turn, determines the cytokines' secretion rate of cells. The value of parameter m has been arbitrarily chosen equal to two.

The bound between IgE and the Fc receptor on mast cells is stable for a number of weeks [24]. Hence we use a negative-binomial distribution for this event, and

we “desensitise” a mast cell with probability p (meaning that the mast cell loses its IgE bound to Fc receptors). Because the expected value of the negative binomial distribution is $1/p$, we set $1/p = 3$ months, that is $p = 0.0037$, given that a time step is 8 hours, as we will see in the following paragraph.

At the end of each time step, cells and immunoglobulins (but not cytokines which purposely are not assumed to diffuse – in order to match the *autocrine* or *paracrine* nature of cytokines signalling [24]) diffuse from one lattice site to a randomly chosen neighbouring site. Each entity moves independently, the whole process resembling a Brownian motion of particles. It should be mentioned that we are taking equal diffusion coefficients for all modelled entities. Clearly this is only a rough approximation. However, this does not really influence the results, since our model assumes a uniform concentration of cells and molecules.

12.3.1 Choosing Parameters

All the model parameter values are given in Table 12.3. This parameter set is considered here the *standard set of parameters*. In other words, parameter values in Table 12.3 are those used in all simulations, unless specified otherwise. Most of the values have been taken from standard immunology literature. The parameters have been grouped as those whose value was taken from known literature, those which are considered arbitrary and those which determine the initial conditions of the simulation. The values of the half-life, τ, are given in Table 12.2.

Antibody molecules as well as cytokines are handled in “quanta” of concentration, that is, there is a minimum amount of molecules which is taken in bulk within the interaction procedures. These quanta are one milligram per millilitre (mg/mL) for antibodies, one femptogram per millilitre (fg/mL) for cytokines and one nanogram per millilitre (ng/mL) for histamine.
Secretion of monoclonal antibodies by hybridomas, in terms of concentration in plasma, is at about 1-20 mg/mL during its lifetime [24]. Therefore we set the antibodies secretion rate in plasma to $b = 10$ μg/mL per time step, which is, 8 hours.

How to set the initial proportion of Th cells in the class 1 or 2 is a major concern. As a matter of fact this proportion is taken as the criterion for distinguishing hypersensitive people from nonhypersensitive ones. In our model, hypersensitive individuals are characterised by having a larger number of initial Th2 cells. We use two parameters for this purpose: α and β. Thus,

$$
\begin{aligned}
Th0(0) &= \alpha Th(0),\\
Th1(0) &= (1-\alpha)\beta Th(0),\\
Th2(0) &= (1-\alpha)(1-\beta)Th(0),
\end{aligned}
$$

where $Th(0)$ is the total number of helper cells (cfr. Table 12.3). The values of α and β, taken arbitrarily here, are given as initial condition and determine the initial level of susceptibility to the allergen.

Table 12.3 Standard parameter set. The initial amount of cytokines (IL-4, IFN-γ, IL-12, T-GF) is set to zero to indicate normal conditions. ($\mathbb{N}$ and $\mathbb{R}$ indicate integers and real numbers respectively.)

Parameter	Meaning	Range	Value
Known values			
b	Ab secretion rate	$\mathbb{N}$	10 (μg/mL/8h)
n	lymphocytes duplication steps	$\mathbb{N}$	5
ρ	cytokines amplif. factor	$\mathbb{N}$	2
Arbitrary parameters			
η	T-GF efficiency	$\mathbb{R}$	50
κ	cytokines inhib. efficiency	$\mathbb{R}$	10
h	histamine secretion rate	$\mathbb{N}$	1 (pg/mL/8h)
Initial conditions			
L	lattice dimension	$\mathbb{N}$	20
$B(0)$	B's init. population	$\mathbb{N}$	260 (cells/mm^3)
$Th(0)$	Th's init. population	$\mathbb{N}$	875 (cells/mm^3)
$MA(0)$	MA's init. population	$\mathbb{N}$	350 (cells/mm^3)
$MC(0)$	MC's initials	$\mathbb{N}$	300 (cells/mm^3)
γ	size constraint	$\mathbb{R}$	10
$1-\alpha$	fraction of Th0	$[0,1)$	0.9
β	fraction of Th1/Th2	$[0,1)$	0.2
ω	cytokines secretion rate	$\mathbb{N}$	100 (0.1pg/mL)

12.3.1.1 Setting the Scale of Time and Space

The time scale of the model is determined by our assumption that a lymphocyte completes one mitosis cycle in one time step. Since, once stimulated, a lymphocyte divides for about three times a day, our time step corresponds to about 8 hours.

Space is not so simple to define in our model. It is the normal adult blood-cell counts which gives us the reference value. In fact, fixing to about 10^3 the initial lymphocytes' counts our simulation space is taken to be about one mm^3 of blood serum. The only arbitrary value is the initial number of mast cells, which is very low in blood, but high in tissues.

Note that when the initial population of cells is fixed, the lattice dimension L determines the concentration, hence the affinity to the antigen and, in general, the interaction probability.

12.4 Model Validation and Simulation Results

In the first set of simulations we are mainly interested in validating the model suitability for retrieving real-life hypersensitivity. To do so, we first check whether the model can reproduce an immune response, while fulfilling the hallmarks of an IgE-mediated hypersensitive reaction in susceptible individuals. Subsequently, a set of simulations (paragraph 12.4.3) are performed to asses the dependence of the IgE level on both the level of IL-4 and that of IFN-γ [59].
The second set of simulations represents the core of our work. Here we investigate the effect of the allergenic drug dosage and administration schedule on the amount of histamine released by mast cells. Finally, in section 12.5, we briefly discuss the main results and suggest some implications for future drug therapy.

It is worth at this point, to spend few words about the way we use the terms "dose" and "concentration" hereafter. When we say "drug concentration" of, e.g., 2000 ng/mL, we actually mean "the dose whose resulting concentration is 2000 ng in a millilitre of blood after a suitable delay of time," since our simulation space is always a millilitre of blood.

12.4.1 Healthy Subjects: Primary and Secondary Immune Response to a Generic Antigen

There are several ways in which *normal* immune response to a generic antigen can be simulated using our model. For instance one can inhibit the production of IL-4 in the model, thus "knocking-out" IL-4 activity [60]. Another possibility is to force Th cells to be of class 1 only (i.e., $\beta = 1$). Here we mimic a healthy subject by using the first method, that is, we set things so that no IL-4 can be released by Th2 cells. The consequence is a bias towards the Th1 response, i.e., a normal immune response.

The drug administration protocol consists of a first injection at initiation (time zero) and a burst injection at day 40. The drug dose for both injections is calculated so that in one $m\mathrm{m}^3$ the concentration is 2000 ng/mL (recall paragraph 12.4). Results suggest that the model system reproduces a classical primary and secondary Th1-type, response (Figure 12.3, panels a, b, and c). In panel (a) we see simulation of the blood levels profiles of the interleukins IFN-γ, IL-4, and IL-12. Note that the first increase in all three interleukins is only marginal, occurring 14 days following the first drug challenge. In contrast, the second drug challenge generates a much more significant response in all the simulated interleukins. The level of T-GF is also different during the first and the second response, but the difference from the hypersensitive case (next paragraph) emerges only at the second injection. Panel (b) shows the level of immunoglobulins produced by plasma cells where the IgM type are eventually overtaken by IgG during the second response. Finally, the system

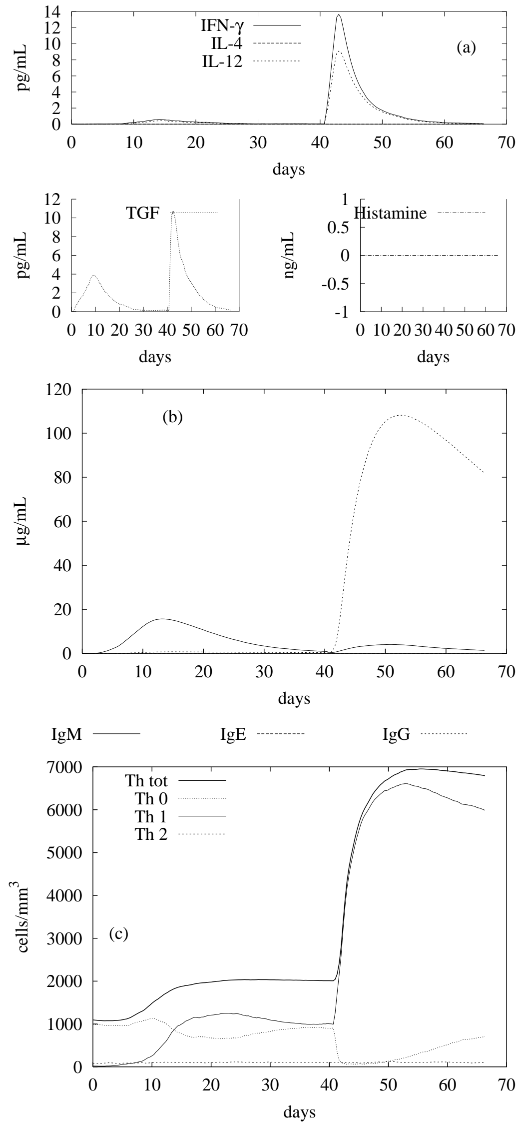

Figure 12.3

Immune response in healthy subjects (or IL-4 knockout mice [60]). Allergenic drug injections are scheduled to be at initiation and in day 40. No histamine is released, because mast cells are not sensitised (not shown) given that no IgEs are secreted (panel (b)). The immune response is of the Th1-type (panel (c)) since IL-4 is absent (panel (a)).

develops immune memory of the Th1-type for the specific antigen (panel (c)).
In summary, using our CA tool we retrieve

- the production of antigen-specific IgG antibody (Figure 12.3, panel (b));
- the production of IFN-γ by type 1 Th cells (Figure 12.3, panel (a)); and
- a moderate degree of proliferation of helper cells (Figure 12.3, panel (c)).

Note that in our "healthy subject" model no IgEs are produced (panel (b)) and therefore mast cells do not get sensitised (panel (a)) and no histamine is released (for simplicity we assumed that the basic level of histamine secretion is null).

12.4.2 Allergic Subjects: Sensitisation and Hypersensitivity to a Generic Allergenic Drug

To simulate hypersensitive subjects, who develop a large amount of immunoglobulins of the IgE isotype, we assume an initial Th1/Th2 ratio (i.e., $\beta = 0.2$). Having made this assumption we expect the course of immune response in the hypersensitive individual to be quite different from that of the normal individual. Letting the simulated drug protocol be the same as in the previous experiment, we obtain results as summarised in Figures 12.4 and 12.5. Figure 12.5b shows the population size of Th1-Th2 cells. Comparing results to those displayed in panel (c) of Figure 12.3 one can immediately notice that the initial bias towards higher proportions of Th2 to Th1 suffices for a Th2-type, or allergic response. This result can be interpreted as follows: the first contact with the allergenic drug triggers Th2 cells to release IL-4 (Figure 12.4a). Consequently, increased production of Th2 cells occurs, resulting from the amplified IL-4 production and the inhibited IL-12 and IFN-γ release (Figure 12.4a). In fact, the inhibition of IL-12 prevents the Th0$\rightarrow$Th1 transition so that the Th0$\rightarrow$Th2 transition is now favoured. Increased proportion of Th2 cells, eventually results in the IgG to IgE isotype switch (Figure 12.4b), and IgEs bound to the Fc receptor on mast cell's membrane are crossed-linked by new allergenic drug molecules to degranulate these cells and release histamine (Figure 12.4a bottom right).

In our model at least *two* bounded IgE molecules are required for a mast cell to become sensitised. Figure 12.5a shows the changes, over time, in the proportion of sensitised mast cells. Note, in this figure, that the counts of sensitised mast cells are lower during the first contact with the allergenic drug. This is because IgM are more numerous at the onset. The second injection dosing of allergenic drug finds the system in the Th2-dominant-state so that a larger production of IL-4 induces a large IgE isotype switch of B lymphocytes (Figure 12.4b) with consequent cross-linking and degranulation of already sensitised mast cells. In summary, note that the first response here is mainly IgM, while the second response is dominated by IgE immunoglobulins. Moreover IgGs are virtually absent during the second response,

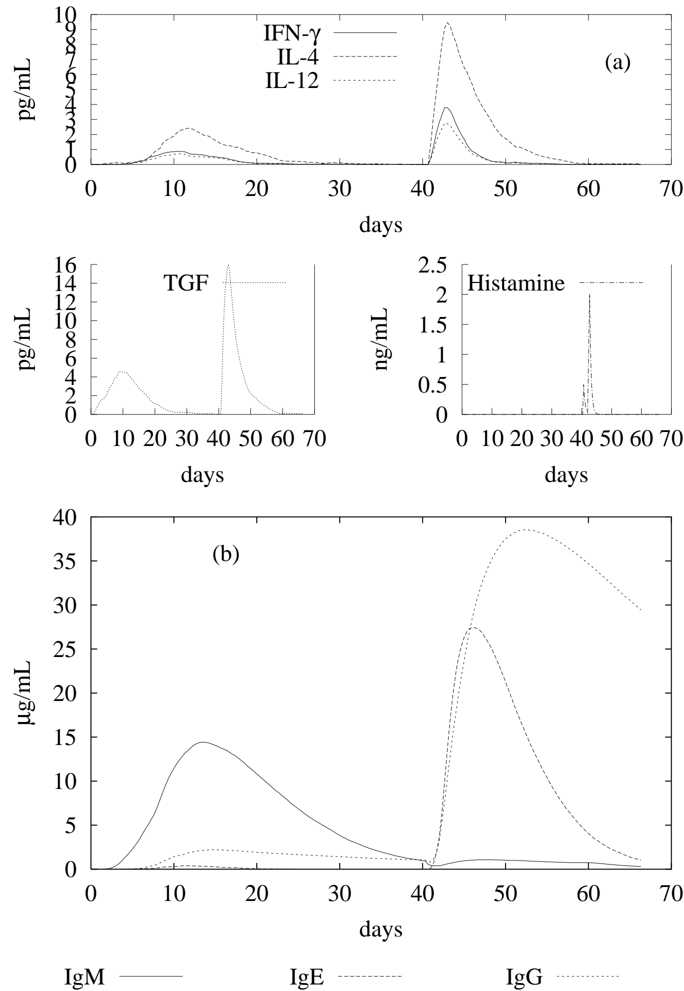

Figure 12.4

Response in hypersensitive subjects. Allergic subjects have a different immune reaction to the allergen compared to healthy (cfr. Figure 12.3). In particular large production of IL-4 which amplify Th0 to Th2 switch (see Figure 12.5) and isotype switch to IgE (panel (b)) with consequent cross-linking (i.e., sensitisation, Figure 12.5) and degranulation with release of histamine (panel (a) bottom right). The parameter $\beta = 0.2$, the injection of allergen is as for Figure 12.3.

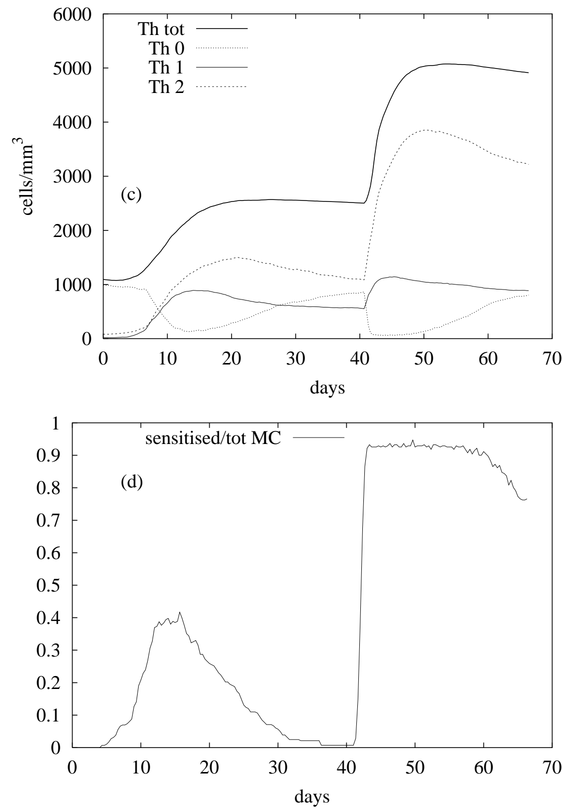

Figure 12.5

Response in hypersensitive subjects. Referring to Figure 12.4, the top panel shows the amplified Th0 to Th2 switch and the bottom panel the sensitisation.

and Th proliferation (Figure 12.5a) is much larger, as compared to that of healthy subjects.

The above simulations validate our model's versatility in reproducing the differences between normal and hypersensitive response.

12.4.3 Effects of IFN-γ and IL-4

To further validate our model we study the effect of IFN-γ and IL-4 on the development of IgE or IgG antibodies (cfr. Figure 12.6). To this end we initiate treatment by injecting a variable amount of IL-4, or a variable amount of IFN-γ. Two weeks later we measure the amount of IgE and IgG produced. Results are shown in Figures 12.6a and 12.6b respectively, where each point is computed averaging the outcome of hundred independent runs.

As expected, the results in Figure 12.6 show that the level of produced IgE is positively correlated with IL-4 dose, since IL-4 not only favours isotype switch to IgE, but also sustains the switch of Th0 to Th2 cells, further producing IL-4 in a positive feedback. In contrast, the level of IgE is negatively correlated with the injected IFN-γ dose, since IFN-γ amplifies the effects of IL-12 released by antigen processing cells, and induces Th0 to undergo a class switch to Th1. In addition, IFN-γ supports isotype switch of B cells to IgG. The inverse results are obtained for the effect of IL-4 and IFN-γ on IgG production. As can be seen in Figure 12.6, our simulation results are in good agreement with real-life observations ([24], observation cited in [59]).

12.4.4 Hypersensitivity Dependence on Drug Dose

In this section we investigate the relationship between the allergenic drug dose and the amount of histamine released. The parameter setting is the same as in the previous experiments, the only difference being the concentration of allergenic drug in blood and the administration schedule.

In these simulations we make use of the knowledge of the "critical histamine level," that is, the amount of secreted histamine above which allergic symptoms appear, whose average value can be defined to be 1 ng/mL [61]. Nevertheless, it should be mentioned here that in clinical practice the distinction between "normal" and "pathologic" is not so sharp, the threshold of hypersensitivity possibly lying anywhere in the interval 1-10 ng/mL.

In the first simulation experiment we check the effects of the dose of the allergenic drug administered at initiation (denoted *sensitisation*). We do so by varying the sensitisation dose and by measuring the peak value of histamine a few days of simulated time following sensitisation. In Figure 12.7a we plot the average peak of histamine measured within few days after the dosing, as computed over a hundred independent simulations. Results show that the level of histamine release increases proportionally to the sensitising allergen dose. Moreover, a sharp increase above the critical allergic threshold of 1 ng/mL histamine concentration is found for drug dosing corresponding to about $10^3 ng/m$L (cfr. Figure 12.7a) .

Hence a *large dose* of drug, administered in a single shot, is not advisable. Therefore a different method of administration should be considered. This can be,

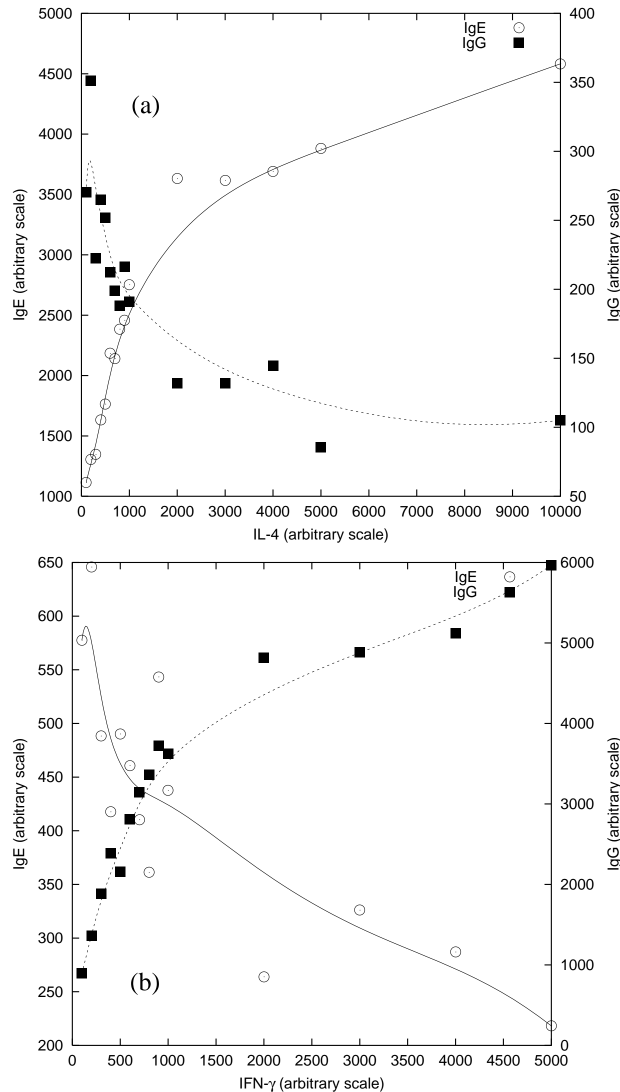

Figure 12.6

IgE and IgG production as a function of the injected IL-4 and IFN-γ doses. IgE production is positively correlated with the injected IL-4 dose and negatively correlated with the dose of IFN-γ. The opposite holds for IgG production. Comparison with *in-vitro* observations is shown (from [24], observation cited in [59]). Concentrations on both axes are given in arbitrary units.

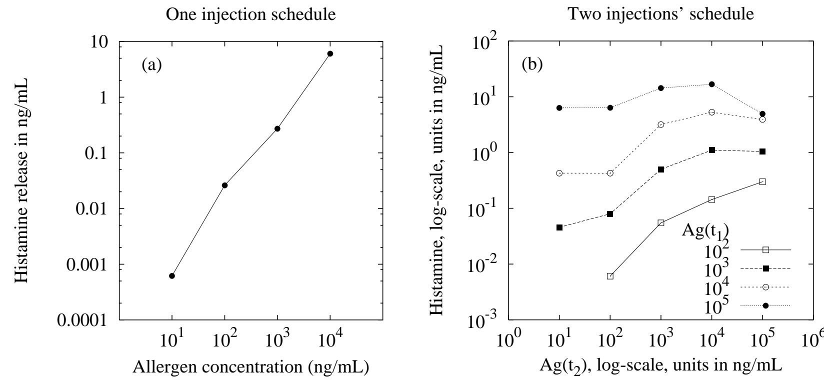

Figure 12.7

Panel (a): Histamine release as a function of the sensitising allergenic drug dose for a single administration. Different doses of allergen are administered at time zero. The corresponding histamine release level increases above the allergic threshold of 1 ng/mL [61] for an allergenic drug dosing corresponding to the critical concentration of $10^3 ng/m$L. One hundred independent simulations are averaged; standard deviation is shown as error bars. Panel (b): Histamine release following two allergenic drug dosings of different doses. The plot shows the histamine concentration during the secondary immune response as a function of drug dose in the second dosing $Ag(t_2)$.

for example, administration in divided doses. In order to use our model for studying the effect of such multi-dosing protocols we make another set of experiments.

These simulations are performed according to the following settings: at time 0 we inject the allergenic drug, dose being $Ag(t_1) = 10^2$, 10^3, 10^4, and 10^5 ng/mL. About two weeks later we administer the same variable amount $Ag(t_2)$ (what is called the *challenge*). Thus, in two administrations we check sixteen different dose-schedules $(Ag(t_1), Ag(t_2))$. One hundred independent simulations are performed for each schedule, and the amount of histamine at the peak level *after the second dosing* is measured. Averages are then computed and plotted in Figure 12.7b.

Results in Figure 12.7b suggest a correlation between the first administration dose $Ag(t_1)$ and the amount of histamine released by mast cells. Thus, a sensitisation dose of $Ag(t_1) = 10^4$ ng/mL risks to stimulate an above-threshold release of histamine, if followed, two weeks later, by a successive dosing above 10^3 ng/mL. It is also interesting to note that a strong sensitisation dosing ($Ag(t_1)$) sets the system to release high levels of histamine, for any positive challenge dose $A(t_2)$ (the points for $Ag(t_1) = 10^5$ remain the same on the log-scale x-axis). This suggests that a larger second dosing is not advisable unless the sensitisation is made with low dosage. In fact, only $Ag(t_1) = 10^2$ and 10^3 ng/mL remain below the critical level

Table 12.4 Parameters estimated from simulated data shown in Figure 12.8 for Equation (12.9).

Allergen concentration (ng/mL)	a	b	c	d
1000	0.899	3.754	2.662	0.215
2000	2.778	3.422	2.676	0.377
5000	7.330	2.701	2.718	0.715

of 1 ng/mL of histamine after the challenge for the doses tried in this experiment. This result is in line with experimental practice during immunotherapies [2,62].

12.4.5 Dependence of Hypersensitivity on Dosing Interval

In the above experiments we used schedules of two allergenic drug cycles, where the second drug administration was constantly delayed by two weeks. In this section we investigate how the histamine level may change under different intervals between the two cycles $\Delta = t_2 - t_1$. The total drug concentration in a $m\text{m}^3$ in both the sensitisation and the challenge dosing ($Ag(t_1)$ and $Ag(t_2)$, respectively) is set to 1000, 2000, or 5000 ng/mL. We first inject the allergenic drug at time zero and for the second time following a variable delay ranging from 3 days to 100 days. As usual, 100 independent simulations are performed for each setting and the amount of histamine at peak level is measured. Averages are then computed and plotted in Figure 12.8.

Histamine secretion as a function of the dosing interval $H(\Delta)$ is well described by the exponential function

$$H(\Delta) = a \exp[-b(\ln(\Delta) - c)^2] + d, \tag{12.9}$$

with parameters given as in Table 12.4.

Some of the parameters can be assigned a biological meaning: a depends on the secretion rate of histamine from mast cells (it is expressed in ng/mL/days; cfr. parameter h in Table 12.3), b is a free parameter, whereas d (expressed in ng/mL) determines the asymptotic level of histamine and may be an important estimation of the level of tolerance. Note that the asymptotic value d strongly depends on the half-life of the immune memory. In fact, if memory fades away before the second dosing, a similar response to the first dosing is to be expected.

A most interesting result is obtained when one attempts to estimate the parameter c (expressed in days). This parameter determines the interval, corresponding to the maximum of the function in Equation (12.9), which is attained at $\Delta = e^{\,c}$. In all cases simulated here this value corresponds to about two weeks, regardless of the allergenic drug dose. Therefore our model predicts two weeks as the delay one should avoid when setting protocols for chemotherapy, regardless of the drug dosage; how far one should deviate from the two-week delay is a function of the dose (see below).

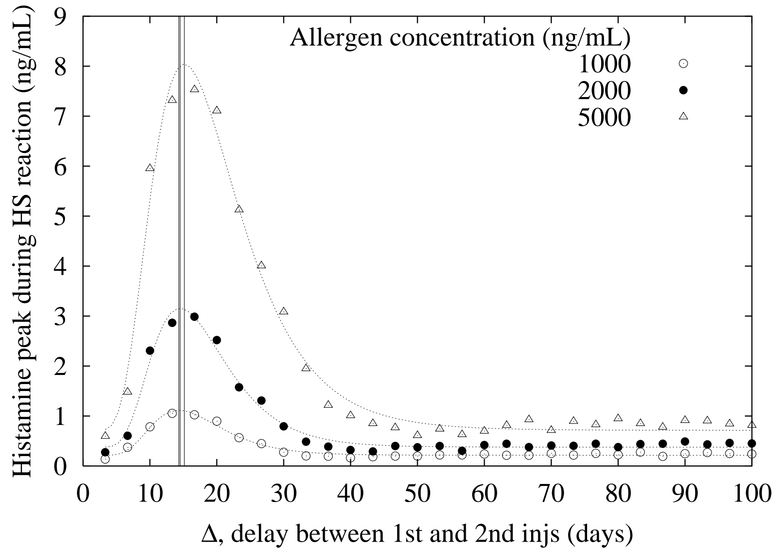

Figure 12.8

Histamine concentration versus $\Delta = t_2 - t_1$. Three regimes are visible: 1) the immune response is still active and wipes out the allergen; 2) large response; and 3) low response because of the decreased number of sensitised mast cells. This stems from the fact that IgE-Fc bound to mast cells can last for e.g., 12 weeks. The 1000 $ng/m\mathrm{L}$ drug-dosage is safe for this two dosings schedule. In contrast, for the 2000 and 5000 $ng/m\mathrm{L}$ drug-dosages there exists a window of durations of the dosing interval, $\Delta_1 - \Delta_2$ (see below), between the two administrations, which increases histamine release above the critical level of 1 $ng/m\mathrm{L}$.

From the simulations in section 12.4.4 we already learned that a safe value for the drug dose would be of 10^3 $ng/m\mathrm{L}$, while 10^4 $ng/m\mathrm{L}$ risks to cause an allergic reaction. Moreover, we already know that for intermediate drug doses there exists a window, for the delay Δ, for which histamine reaches levels above the critical value of 1 $ng/m\mathrm{L}$ which we call now $\tilde{h}$. Performing some simple algebra, using Equation (12.9), we may compute the width of the window as $\Delta_2 - \Delta_1$, where

$$\Delta_1 = \exp\left(c - \sqrt{\frac{1}{b}\ln\frac{a}{\tilde{h} - d}}\right),$$

and

$$\Delta_2 = \exp\left(c + \sqrt{\frac{1}{b}\ln\frac{a}{\tilde{h} - d}}\right),$$

for $a > \tilde{h} - d$ and $d \neq \tilde{h}$. Hence, for example, after estimating the coefficient of the function in Equation (12.9), we can predict whether or not a certain time-schedule

protocol is advisable. For example, for the case of 5000 ng/mL drug administration, we obtain $\Delta_1 = 5.06$ days and $\Delta_2 = 45.33$ days.

12.4.6 Fractionating the Drug Dose into Multiple Dosings

We now consider the question whether hypersensitivity reaction can be avoided by further fractionating each drug dosing within a therapy-cycle in a manner equivalent to "applying slow drug administration." In order to address such an issue we perform a set of experiments in which two therapy-cycles are composed of a period of administration of one, two, or three time steps each. Recall that a time step is 8 hours, so that one dosing is equivalent to an 8-hour period of infusion, two dosings are equivalent to a 16-hour period of infusion, and so on. As usual, 100 runs for each scenario are performed, peaks of histamine are measured and averages are shown in Figure 12.9. Plot (a) refers to the total administration of 1000 ng/mL, plot (b) to 3×10^3, and (c) refers to 10^4. As shown, for the case (a) and (b), there is no much difference in the hypersensitivity reaction which results from single or multiple drug dosing of the same total dose. A more significant difference is seen in plot (c), but this is probably due to statistical fluctuations of the results. At this time, given the gross time-resolution adopted in the model (8 hours per time step) we are unable to say if such a result is due to some systematic phenomenon.

12.5 Discussion

Many antitumour drugs have been shown to induce hypersensitive responses. One of the extensively studied examples is the widely used cytotoxic drug, paclitaxel, which is administered in a variety of solid tumour diseases [21,63]. As hypersensitivity to the drug can hamper further therapy, it may be interesting to investigate the effects of the allergenic drug schedule, dose fractionation, dosing interval, and rate of administration, on the resulting symptoms of hypersensitivity. To study these questions we simulated various drug treatment scenarios, employing a CA model of the immune system. Our results are interpreted below hoping to arrive at some practical conclusions for future therapies.

We started by studying the dose effect of a single allergenic drug administration. Figure 12.7a shows that there exists a critical value for the amount of allergenic drug dose for which the allergic threshold of 1 ng/mL of histamine released is exceeded. This critical concentration is found to lie between 10^3 and 10^4 ng/mL.

We then considered the fractionation of a single dosing into two administration cycles consisting of a single dosing each, and separated by a considerable fixed interval (two weeks). For investigating the effect of the dosage in a schedule of two successive cycles, different schedules are chosen for the simulations reported in Fig-

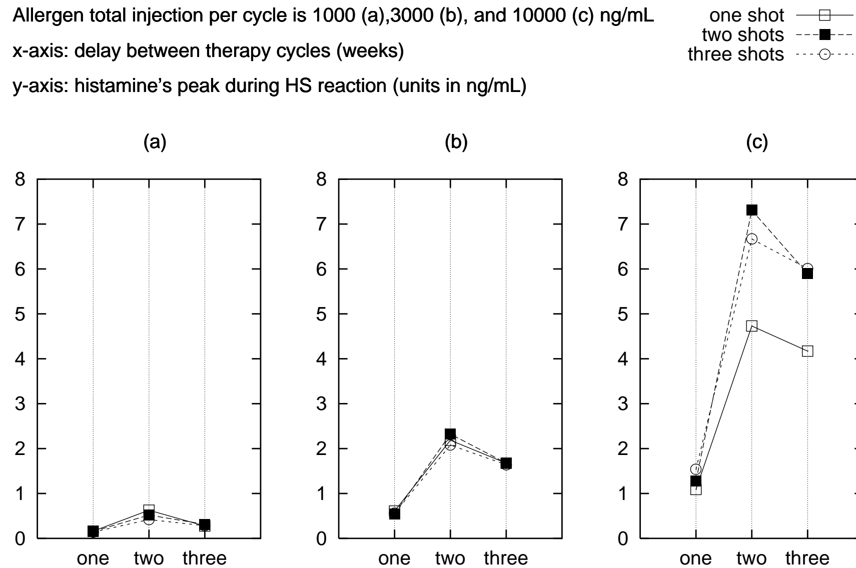

Figure 12.9

Histamine release corresponding to different slow drug administration protocols: two dosing cycles performed at day zero and after one, two, or three weeks. At each cycle, the dosing is performed over a period of 8, 16, or 24 hours. The total amount of allergenic drug is 10^3 $ng/m\mathrm{L}$ in plot (a), 3×10^3 in plot (b), and 10^4 in plot (c). The amount of allergenic drug is equally divided for the period of drug administration, so that, for example, the 16 hour administration in case (a), corresponds to two consecutive dosings (after 8 hours) of 0.5×10^3 $ng/m\mathrm{L}$.

ure 12.7b. Interestingly, we found that the drug dose during the second cycle does not significantly influence the histamine level released by mast cells, if the sensitisation dosing, that is the dosing of the first cycle, is high. The most straightforward explanation for this result is that, due to strong sensitisation, a large number of antibodies are formed, which actually compete for the allergen during the challenge with the sensitised IgE-bound mast cells. Critical concentration of histamine is reached for high drug dosage (even for 10^4 $ng/m\mathrm{L}$), if the challenge is a large dose (for example above 10^3 $ng/m\mathrm{L}$). Note that this is in agreement with the clinical experience in immunotherapeutic protocols: one starts with a small amount of allergen and gradually increases the amount to reach a certain maximum [2]. Indeed, in Figure 12.7b one can easily see that, for equal total drug dose (first dosing + second dosing), the histamine released during the challenge is higher when the first dosing is larger and, vice versa, it is lower for lower first dosing.

The second issue to be discussed is the effect of the allergenic drug dosing interval on hypersensitivity. Replacing the fixed dosing interval of two weeks, we

simulated cycles of therapy with delays of variable number of days between them. The results, summarised in Figure 12.7b, single-out a dosing interval of two weeks as the centre of the peak in histamine release, regardless of the dose of drug injected. The histamine increases above the critical pathological level only if the two cycles are critically distanced, but not if they are very shortly distanced. However, when using a larger amount of drug, both the boundaries of the critical window expand, leftward and rightward reducing the chances for an optimal delay between cycles.

A final question arises when considering prolongation of the drug administration period within a single cycle (i.e., one or more shots). In order to address such an issue we performed a set of experiments in which a therapy-cycle is composed either by a single, two, or three shots. Results are those shown in Figure 12.9. As suggested by this figure, the rate of drug administration of the same total dose has no or little effect on hypersensitivity, if the administered dose is one or three μg/mL. For higher dosage (panel (c)) some effect arises but given the large time scale adopted in our model (8 hours/time step) we are unable to tell more about it.

In summary, our results suggest that in order to avoid allergic reactions to drugs during therapeutic anti-cancer treatments, one should administer a growing dosage of drugs in the same way allergen is administered to the patient during immunotherapies. The interval between successive cycles should be either very short (if the drug dose is low) or very long (if the drug dosage is high) while the question if prolonged period of infusion reduces the risk of allergic reaction remains unanswered.

12.6 Conclusions

Simulations validate the ability of the model to capture the basics of immune phenomena in both normal and drug-hypersensitive individuals. By means of extensive numerical simulations we observe a strong correlation between histamine release and both allergenic drug dosage and the interval between successive dosings.

Varying the interval between successive drug dosings we find that histamine release after the burst dosing has a peak around e^c = two weeks, where c is a constant which seems independent of the allergenic drug dose. This result suggests the existence of an optimal value to be used during anti-cancer therapies.

Our results, however, may be improved in two different directions.

1. At present, we considered only mast cells to be responsible for the allergic reaction. However, it is believed that eosinophils provide further stimulation to the mast cells, inducing degranulation during the late-phase response, which occurs some minutes after the early phase [56]. Therefore, a possible improvement of the model would be to take them into account in order to draw more accurate conclusions.

2. In addition, a finer-grain in the definition of the time-resolution may aid in verifying whether increasing the period of drug administration, within each single

therapeutic cycle may reduce, or, rather, increase the magnitude of hypersensitive reaction.

Acknowledgments

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12.7 References

[1] Weiss, R.B., Hypersensitivity reactions, *Semin. Oncol.* 19, 458-477, 1992.

[2] Kay, A.B., Allergy and allergic diseases. First Part, *N. Engl. J. Med.* 344, 30-37, 2001.

[3] Agur, Z., Arnon, R., and Schechter, B., Reduction of cytotoxicity to normal tissues by new regimes of phase-specific drugs, *Math. Biosci.* 92, 1-15, 1988.

[4] Cojocaru, L. and Agur, Z., Theoretical analysis of interval drug dosing for cell-cycle-phase-specific drugs, *Math. Biosci.* 109, 85-97, 1992.

[5] Agur, Z. and Dvir, Y., Use of knowledge on $\{\phi_n\}$ series for predicting optimal chemotherapy treatment, *Random & Comp. Dyn.* 2, 279-286, 1994.

[6] Webb, G.F., Resonance phenomena in cell population chemotherapy models, *Rocky Mountain J. Math.* 20, 1195-1216, 1990.

[7] Johnson, M. and Webb, G.F., Resonances in age structured cell population models of periodic chemotherapy, *Int. J. Appl. Sci. Comp.* 3, 57-67, 1996.

[8] Dibrov, B. et al., Mathematical model of cancer chemotherapy. Periodic schedules of phase-specific cytotoxic-agent administration increasing to selectivity of therapy, *Math. Biosci.* 73, 1-31, 1985.

[9] Agur, Z. et al., Zidovudine toxicity to murine bone marrow may be affected by the exact frequency of drug administration, *Exp. Hematol.* 19, 364-368, 1991.

[10] Agur, Z., Arnon, R., and Schechter, B., Effect of the dosing interval on survival and myelotoxicity in mice treated by cytosine arabinoside, *Eur. J. Cancer* 28A, 1085-1090, 1992.

[11] Agur, Z. et al., AZT effect on the bone marrow - a new perspective on the Concorde trials, *J. Biol. Sys.* 3, 241-251, 1995.

[12] Ubezio, P. et al., Increasing 1-b-D-arabinofuranosylcytosine efficacy by scheduled dosing intervals based on direct measurement of bone marrow cell kinetics, *Cancer Res.* 54, 6446-6451, 1994.

[13] Holgate, S.T., Allergic disorders, *British Med. J.* 320, 231-234, 2000.

[14] Ilachinski, A., *Cellular Automata: A Discrete Universe*, World Scientific Publishing Company, London, Singapore, 2001.

[15] Celada, F. and Seiden, P.E., A computer model of cellular interaction in the immune system, *Immun. Today* 13, 56-62, 1992.

[16] `http://www.iac.rm.cnr.it/~filippo/cimmsim.html`

[17] Aulbert, E. and Schmidt, C.G., Anaphylactic reaction in cyclophosphamide infusion, *Onkologie* 6, 82-83, 1983.

[18] Sakura, H. et al., Occurrence of an anti-peplomycin IgE antibody cross-reacting with bleomycin in a patient with cervical uterine cancer, *Cancer Chemother. Pharmacol.* 23, 333-336, 1989.

[19] Szepfalusi, Z. et al., IgE-mediated allergic reaction to hyaluronidase in paediatric oncological patients, *Eur. J. Pediatr.* 156, 199-203, 1997.

[20] BC Cancer Agency drug database. `http://www.bccancer.bc.ca/HPI/ /DrugDatabase/DrugIndexALPro/Paclitaxel.htm`

[21] Essayan, D.M. et al., Successful parental desensitization to paclitaxel, *J. All. Clin. Immun.* 97, 42-46, 1996.

[22] Kay, A.B., Allergy and allergic diseases. Second Part, *N. Engl. J. Med.* 344, 109-113, 2001.

[23] Gell, P.G.H. and Coombs, R.R.A., *Clinical Aspects of Immunology*, 1st Edition, Blackwell Scientific Publications, Oxford, 1963.

[24] Goldsby, R.A., Kindt, T.J., and Osborne, B.A., *Kuby Immunology*, 4th Edition, W.H. Freeman and Company, San Francisco, 2000.

[25] Barnes, K.C. and Marsch, D.G., The genetics and complexity of allergy and asthma, *Immunol. Today* 19, 325-332, 1998.

[26] Mossman, T.R. and Coffman, R.L., Th1 and Th2 cells: different patterns of lymphokine secretion lead to different functional properties, *Annu. Rev. Immunol.* 7, 145-173, 1989.

[27] Bergman, C., van Hemmen, L.J., and Segel, A.L., Th1 or Th2: how an appropriate T helper response can be made, *Bull. Math. Biol.* 63, 405-430, 2000.

[28] Kips, J.C. et al., Interleukin-12 inhibits antigen-induced airway hyperresponsiveness in mice, *Am. J. Respir. Crit. Care Med.* 153, 535-539, 1996.

[29] Magnan, A. et al., Venom immunotherapy induces monocyte activation, *Clin. Exp. Allergy* 31, 1303-1339, 2001.

[30] Perelson, A. and Weisbuch, G., Immunology for physicists, *Rev. Mod. Phys.* 69, 1219-1267, 1997.

[31] de Oliveira, S., de Oliveira, P., and Stauffer, D., *Evolution, Money, War, and Computers - Non-Traditional Applications of Computational Statistical Physics*, Teubner, Stuttgart, 1999.

[32] Bell, G.I., Mathematical model of clonal selection and antibody production, *J. Theor. Biol.* 29, 191-232, 1970.

[33] Bell, G.I., Mathematical model of clonal selection and antibody production II, *J. Theor. Biol.* 33, 339-378, 1971.

[34] Segel, L.A. and Bar-Or, R.L., On the role of feedback in promoting conflicting goals of the adaptive immune system, *J. Immunol.* 163, 1342-1349, 1999.

[35] Antia, R., Pilyugin, S.S., and Ahmed, R., Models of immune memory: on the role of cross-reactive stimulation, competition, and homeostasis in maintaining immune memory, *Proc. Natl. Acad. Sci. USA* 95, 14926-14931, 1998.

[36] McLean, A.R., Modelling T cell memory, *J. Theor. Biol.* 170, 63-74, 1994.

[37] Marchuk, G.I., Romanyukha, A.A., and Bocharov, G.A., Mathematical model of antiviral immune response. II. parameters identification for acute viral hepatitis B, *J. Theor. Biol.* 151, 41-69, 1991.

[38] Marchuk, G.I. et al., Mathematical model of antiviral immune response. I. data analysis, generalized picture construction and parameters evaluation for hepatitis B, *J. Theor. Biol.* 151, 1-40, 1991.

[39] Nowak, M.A. and May, R.M., Mathematical biology of HIV infections: antigenic variation and diversity threshold, *Math. Biosci.* 106, 1-21, 1991.

[40] Perelson, A.S. and Nelson, P.W., Mathematical analysis of HIV-1 dynamics *in vivo*, *SIAM Review* 41, 3-44, 1999.

[41] Kirschner, D.E., Using mathematics to understand HIV immune dynamics, *Notices Amer. Math. Soc.* 43, 191-202, 1996.

[42] Kirschner, D.E. and Webb, G.F., A model for treatment strategy in the chemotherapy of AIDS, *Bull. Math. Biol.* 58, 367-391, 1996.

[43] Perelson, A.S. and Oster, G.F., Theoretical studies of clonal selection: minimal antibody repertoire size and reliability of self-non-self discrimination, *J. Theor. Biol.* 81, 645-670, 1979.

[44] Agur, Z., Mazor, G., and Meilijson, I., Maturation of the humoral immune response as an optimization problem, *Proc. R. Soc. Lond. B Biol. Sci.* 245, 147-150, 1991.

[45] Agur, Z., Mazor, G., and Meilijson, I., *Mimicking the strategy of the immune system: insight gained from mathematics. Theoretical and Experimental Insights into Immunology*, A.S. Perelson and G. Weisbuch, Eds., Springer-Verlag, Heidelberg, 1992.

[46] Agur, Z., Resilience and variability in pathogens and hosts, *IMA J. Math. Appl. Med. Biol.* 4, 295-307, 1987.

[47] Celada, F. and Seiden, P.E., Affinity maturation and hypermutation in a simulation of the humoral immune response, *Eur. J. Immunol.* 26, 1350-1358, 1996.

[48] Seiden, P. and Celada, F., A model for simulating cognate recognition and response in the immune system, *J. Theor. Biol.* 158, 329-357, 1992.

[49] Morpurgo, D. et al., Modelling thymic functions in a cellular automaton, *Int. Immunol.* 7, 505-516, 1995.

[50] Kohler, B. et al., A systematic approach to vaccine complexity using an automaton model of the cellular and humoral immune system, *Vaccine* 19, 862-876, 1999.

[51] Bernaschi, M. and Castiglione, F., Evolution of an immune system simulator, *Comp. Biol. Med.* 31, 303-331, 2001.

[52] Jenmalm, M.C. et al., Allergen-induced Th1 and Th2 cytokine secretion in relation to specific allergen sensitization and atopic symptoms in children, *Clin. Exp. Allergy* 31, 1528-1535, 2001.

[53] Arshad, S.H. and Holgate, S., The role of IgE in allergen-induced inflammation and the potential for intervention with humanized monoclonal anti-IgE antibody, *Clin. Exp. Allergy* 31, 1344-1351, 2001.

[54] Wills-Karp, M., Interleukin-12 as a target for modulation of the inflammatory response in asthma, *Allergy* 55, 113-119, 1998.

[55] Hayes, M.P., Wang, J., and Norcross, M.A., Regulation of interleukin-12 expression in human monocytes: selective priming by interferon-γ of lipopolysaccharide-inducible p35 and p40 genes, *Blood* 86, 646-650, 1995.

[56] Piliponsky, A.M. et al., Human eosinophils induce histamine release from antigen-activated rat peritoneal mast cells: a possible role for mast cells in late-phase allergic reactions, *J. Allergy Clin. Immunol.* 107, 993-1000, 2001.

[57] Agur, Z., Daniel, Y., and Ginosar, Y., The universal properties of stem cells as pinpointed by a simple discrete model, *J. Math. Biol.* 44, 79-86, 2002.

[58] Slifka, M.K. et al., Humoral immunity due to long-lived plasma cells, *Immunity* 8, 363-372, 1998.

[59] Del Prete, G. et al., IL-4 is an essential factor for the IgE synthesis induced *in vitro* by human T cell clones and their supernatants, *J. Immunol.* 140, 4193-4198, 1988.

[60] Kuhn, R., Rajewsky, K., and Muller, W., Generation and analysis of interleukin-4 deficient mice, *Science* 254, 707-710, 1991.

[61] Renz, C.L. et al., Tryptase levels are not increased during vancomycin-induced anaphylactoid reactions, *Anesthesiology* 89, 620-625, 1998.

[62] Secrist, H., DeKruyff, R.H., and Umetsu, D.T., Interleukin 4 production by CD4+ T cells from allergic individuals is modulated by antigen concentration and antigen-presenting cell type, *J. Exp. Med.* 181, 1081-1089, 1995.

[63] Dorr, R.T., *The Annals of Pharmacotherapy*, Harvey Whitney Books Company, Cincinnati, OH, 1994.