Implementation of a regulatory gene network to simulate the TH1/2 differentiation in an agent-based model of hypersensitivity reactions

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ABSTRACT

Motivation: An unbalanced differentiation of T helper cells from precursor type TH0 to the TH1 or TH2 phenotype in immune responses often leads to a pathological condition. In general, immune reactions biased toward TH1 responses may result in auto-immune diseases, while enhanced TH2 responses may cause allergic reactions. The aim of this work is to integrate a gene network of the TH differentiation in an agent-based model of the hypersensitivity reaction. The implementation of such a system introduces a second level of description beyond the mesoscopic level of the inter-cellular interaction of the agent-based model. The intra-cellular level consists in the cell internal dynamics of gene activation and transcription. The gene regulatory network includes genes-related molecules that have been found to be involved in the differentiation process in TH cells.

Results: The simulator reproduces the hallmarks of an IgE-mediated hypersensitive reaction and provides an example of how to combine the mesoscopic level description of immune cells with the microscopic gene-level dynamics.

Availability: The basic version of the simulator of the immune response can be downloaded here: http://www.iac.cnr.it/~filippo/C-lmmSim.html

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Supplementary information: Supplementary data are available at Bioinformatics online.

1 Introduction

In this study we construct a multi-level model of the hypersensitivity response accounting for both mesoscopic (inter-cellular) description of the immune response to a generic allergen and the microscopic (intra-cellular) events related to cell signaling, gene expression and receptor activation, that is involved in the differentiation of CD4* T helper lymphocytes (TH).

We combine two different previous works: the first is an agent-based model of the type I hypersensitivity reactions that has shown to be able to reproduce the hallmarks of the response to a generic allergen, including TH phenotype and IgE isotype switch (Castiglione et al., 2003); the second is an implementation of a gene regulatory network for the switch of TH1/2 reconstructed from published experimental data (Mendoza, 2006). The stable states of that network are consistent with stable molecular patterns of activation of TH0, TH1 and TH2.

In the following sections we give some background information on hypersensitivity reactions, then we describe the model and finally we discuss the simulation results.

1.1 Background on hypersensitivity

The term atopic refers to people suffering some 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. 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 industrialized countries, revealing a link between modern style of life and atopy (Kay, 2001a).

In recent decades, scientists, clinicians and epidemiologists have elucidated the intra-cellular and the cellular mechanisms involved in allergic reactions, including the roles of TH helper subsets and interleukins (Goldsby et al., 2000). 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 sensitizing route, sometimes an adjuvant, and most importantly, the genetic constitution of the recipient (Kay, 2001a,b; Holgate, 2000).

Acute or immediate type I hypersensitivity reaction is a consequence of mediators (histamine, leukotrienes, prostaglandin, etc.) released by mast cells (MCs) or basophils triggered via the allergen-mediated cross-linking of cell surface bound
immunoglobulin-E (IgE). Convincing evidence has accumulated, suggesting that the immune response to allergens in atopics is biased towards the T helper type II (TH2) phenotype, characterized 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 (Goldsby et al., 2000). Allergic IgE responses occur mainly on mucous membrane surfaces in response to allergens (i.e. common environmental antigens), which enter the body either by inhalation or ingestion. Typically, such responses manifest themselves in systemic anaphylaxis, localized or hay fever, asthma, hives, food allergies and eczema. 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 sulfonamides, local anesthetics. 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 protein derivatives etc. (Goldsby et al., 2000).

Immunology textbooks and review articles agree on few basic events culminating with a type I hypersensitivity reaction. We have built on these to implement our mesoscopic scale model of the phenomena: (i) the allergen triggers a primary (biased) TH2 response (i.e. production of TH2 cytokines); (ii) IL-4 induces the isotype switch in B cells and inhibits TH1 activation; (iii) plasma B cells originate from stimulated B cells develop antibodies of the IgE type; (iv) IgEs bind to Fc receptors on MCs (sensitization phase); (v) IgEs bound to MCs bind new encountered allergen (allergic reaction phase) and cross-link Fc-IgE complexes and (vi) cross-linking on MCs causes degranulation.

In the next sections we give a more detailed description of the processes that constitutes the key features of our work, namely isotype switch and TH1/2 differentiation for which we build a gene-regulatory network.

1.2 Immunoglobulins and the isotype switch

During the primary response of a normal (i.e. non-atopic) 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 catabolized and broken down. Should the same pathogen attempts to re-invade the body, it will stimulate a faster and stronger antibody production (it is the secondary response). 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 (i.e. non-atopic) individual is altered in atopics, 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 (Goldsby et al., 2000). 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 MCs and basophils through the Fc receptor on the cell membrane, thus sensitizing these cells. A subsequent exposure to the same allergen, induces cross-linking of IgE-bound molecules on sensitized 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, neutrophil chemotactic factor, platelet-activating factor, leukotrienes, prostaglandin, etc. Finally, the presence of these active molecules triggers 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) (Goldsby et al., 2000; Barnes and Marsch, 1998).

1.3 Cytokines production and the role of TH1/2 differentiation

T helper lymphocytes are mainly classified according to the types of cytokines they secrete (Mossman and Coffman, 1989). 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 intra-cellular 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.

Together with TH lymphocytes, the macrophages (MA) are the main source of the different interleukins. Among others, they secrete IL-12 and IL-18 which induce 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 (Goldsby et al., 2000). The interleukin IL-12 and IL-18, promote TH1 lymphocyte’s enhanced secretion of IFN-γ. Conversely, IFN-γ promotes IL-12 and IL-18 secretion so that there is a positive feedback between these cytokines (Fig. 1). In contrast, by driving the TH response to the TH1 phenotype, IFN-γ acts as a suppressive agent of the allergic immune response (Kips et al., 1996, Magnan et al., 2001). On the other hand, IL-4 is identified as a pro-TH2 interleukin.

2 METHODS

2.1 Multi-scale simulator

The model at the mesoscopic scale is based on the clonal selection theory of F.M. Burnet developed on the tracks first highlighted by P. Ehrlich at the beginning of the 20th century.
Seiden, 1992). Specialized versions of the same basic model to simulate previous publications (Bernaschi and Castiglione, 2001; Celada and Jenmalm et al., 2005). The version we employ here includes mechanisms related to TH differentiation, isotype switch and histamine release. A cubic millimeter of blood serum is mapped onto a 2D hexagonal lattice (six neighbors), with periodic boundary conditions. Physical proximity is modeled through the concept of lattice-site. All interactions among cells and molecules take place within a lattice-site in a single time step corresponding to 8 h of real life (this will be discussed later). At the end of each step, entities move to adjacent lattice sites to model cellular and molecular mobility introducing spatial correlations.

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 MCs) are represented. Cells are added through an external compartment, which modeled through positive and negative selection of immature thymocytes before they get into the lymphatic system (Morpurgo et al., 1995).

The interactions among cells and molecules determine their functional behavior. They may be a-specific (e.g. antigen phagocytosis by monocytes or macrophages, binding by MCs, etc.) or specific according to their affinity or degree of chemical binding strength (e.g. TH interacting with B cells for antigen recognition, etc.).

At each time step of the simulation 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.

Among the multitude of cytokines involved in an immune response only a subset is taken into account in the present model (Fig. 1 shows the cytokines involved in the TH differentiation). These are the ones which are directly involved in the allergic reactions (Jenmalm et al., 2001; Arshad and Holgate, 2001) as follows:

- Interleukin-2 (IL-2), is secreted by stimulated T helper cells. IL-2 is also known as T-cell growth factor (TCGF). It promotes clonal expansion and differentiation of T helper and B cells (this is not shown in Fig. 1 since it is not involved in TH differentiation);
- Interleukin-4 (IL-4), stimulates antibody-producing B-cells to produce IgE instead of IgG. IL-4 inhibits IL-12 released by macrophages and TH1 proliferation and promotes TH2 clone expansion;
- Interleukin-12 (IL-12), acts in a contrasting manner to IL-4, promoting TH1 type response and strongly stimulating T cells to synthesize IFN-γ (Wills-Karp, 1998; Magnan et al., 2001);
- Interleukin-18 (IL-18), has the same effect of IL-12 but is produced by macrophages and not by B cells (Akira, 2000);
- Interferon-γ (IFN-γ), is secreted by TH1 cells and induces antibody switch to IgG. It also stimulates IL-12 production (Hayes et al., 1995; Magnan et al., 2001) so that there is a positive feedback between IFN-γ and IL-12 (Fig. 1).

The autocrine and paracrine 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. Moreover we make the following working assumptions:

- all cells release either the same basic amount (indicated by ω) of cytokine or they secrete an enhanced number (ρ \cdot ω) of cytokine (the enhancement corresponding to doubling the rate of secretion i.e. ρ = 2);
- the basic amount ω is equal for all cytokines. This implies that all cytokines have the same efficacy in exercising their action;
- in order to distinguish hypersensitive subjects from normal ones we use the criterion of setting the initial proportion of TH cells in the class 2, TH2(0), to be higher than those in class 1, TH1(0). We use two parameters for this purpose: α, β ∈ (0, 1). Thus, TH0(0) = αN, TH1(0) = (1 - α)βN and TH2(0) = (1 - α)(1 - β)N where N = TH(0) is the total number of helper cells irrespective of the phenotype. The value of α < 1 is the fraction of TH cells that are already committed whereas β, given as initial condition, determines the initial level of susceptibility to the allergen. β ∼ 0.2 corresponds to an atopic subject.

The definition of space is indirectly given by using the normal adult blood-cell counts as the reference value. In fact, fixing to about 10^8 the initial lymphocytes’ counts our simulation space is taken to be about 1 mm^3 of blood serum.

The time scale of the mesoscopic level 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 h.

The isotype switch occurring to a B cell sitting on lattice point x is modeled as a Bernullian event with probability p given by a sigmoid-Hill function depending on the concentration c_ω of the pro-switch cytokine (recall that IL-4 is pro-TH2 whereas IFN-γ is pro-TH1). That is, p = c_ω/(ω^2 + c_ω^2) where ω is the basic amount of cytokines secreted by cells (see above).

The overall dynamics of the model can be easily summarized as follows: once we inject the antigen, some cells move from the inactive state to the active one, through the interaction with other cells and/or molecules. It follows a cascade of events culminating in the clonal expansion of lymphocytes. In absence of antigenic stimulus the system is in a stable state. In other words, the antigen injection breaks the equilibrium bringing the collective dynamics to a metastable state of infection.

### 2.2 Gene network regulating the differentiation of TH cells

As already mentioned, the switch of T helper cells from the precursor type TH0 into TH1 and TH2 is a major issue to characterize atopic and non-atopic subjects. In Mendoza (2006), a qualitative model for the
regulatory network controlling the TH1/2 differentiation process has been proposed.

Various kinds of molecules (secreted cytokines, receptors, signal transducers and transcription factors), were considered (Fig. 2). Each of these nodes represents a molecule that is known to participate in the differentiation process; the network was built based on published experimental data, all the information comes from in vitro experiments with mouse or human cells (Mendoza, 2006).

The obtained regulatory graph represents the most extensive attempt to model the regulatory network controlling the differentiation of TH lymphocytes to date. The dynamics of the resulting network were analyzed using classical logical method to gain qualitative informations about the dynamical properties of the system (Thomas, 1991; Thomas et al., 1995). In our case, to obtain a dynamics closer to the reality the classical Boolean formalism was extended by allowing the variables to assume three discrete values: low (L), medium (M) and high (H). A regulatory network can be depicted as a directed graph in which nodes represent entities (e.g. secreted cytokines, receptors, signal transducers and transcription factors; see Fig. 2 and Table 1) and edges represent interactions among them (e.g. inhibition, activation, etc.). The overall state of the network is given by the expression levels of all entities. The network state is updated according to rules transcribing the biological influence among entities (i.e. it is a dynamical system) (Supplementary Material S1).

We consider 17 nodes with some little differences with respect to Mendoza (2006): i) in order to distinguish the input nodes from the internal ones (that have a feedback effect) we added two more nodes, namely, ‘IFN-γ input’ and ‘IL-4 input’; ii) node IFN-β and IFN-βR have been omitted since in our model the cytokine IFN-β has overlapping effects with IFN-γ.

Note that the four nodes IFN-γ, IL-4, IL-12, IL-18 that act as input for the regulatory-gene network, connect the dynamics of the the inter-cells level and an intra-cells one.

The transition rules regulating the network were used to study the stable states of the net trough the analysis of circuits and loops. Three ‘attractors’ were identified: two leading to TH1 and one to TH2 (respectively called P1, P2 and P3).

TH1 corresponds to two sub cases:

P1. high levels of activation of IFN-γ, SOCS-1 and T-bet, medium level of IFNγR, and STAT-1, and all other nodes at low level;

P2. medium level of IFN-γ, IFN-γR, STAT-1 and T-bet; a high level of SOCS-1, and all other nodes at low level.

TH2 corresponds to:

P3. high levels of activation of IL-4, IL-4R, STAT-6 and GATA-3, and all other nodes at low level.

In other words, the network activation levels of the nodes corresponding to one of the attractors P1, P2 and P3, determine the commitments of the associated cell to a TH1 or TH2 phenotype. The commitment of a TH0 to the TH1 or TH2 phenotype is definitive.

More formally, we obtained a partition of the space of all possible configurations \( \Omega = \{0, 1, 2\}^7 \) considering hyper spheres of radius \( i = 1, 2, 3 \), where \( a \in \Omega \), \( b \in \Omega \), \( d(a, b) = \sum_{k=1}^{7} |a_k - b_k| \) and \( B_\Omega = \Omega - (B_{P1} \cup B_{P2} \cup B_{P3}) \) is the remaining space. Note that \( B_{P1} \cap B_{P2} \neq \emptyset \) while \( (B_{P1} \cup B_{P2}) \cap B_{P3} = \emptyset \). All TH cells, whose internal network state belong to \( B_{P1} \) and \( B_{P2} \), are marked as TH1; those with internal state in \( B_{P3} \) are marked as TH2 and all the rest as TH0.

After each (macro) time step (that is, the inter-cell dynamics of the agent-based level), each TH0 cell ‘senses’ the concentration of the input cytokines in the same lattice point and sets the activation level of the corresponding input nodes (in the same logic). It follows the internal (molecular) dynamics of the gene network consisting in \( k \) micro steps, during which the activation levels of the nodes change according to the transition rules (Supplementary Material S1). The final state is analyzed to decide the transition of the cell to the TH1 or TH2 phenotype. If no transition occurs (because for example the network state is not in P1 or P2) then the cell remains in that state and the micro dynamics at the next iteration starts from there. It is worth to point out that the activation level of the input nodes does not change during the \( k \) micro steps.

The overall multi-level simulation consists in alternating a single macro step (of the agent-based inter-cellular level) with \( k \) micro steps (of the intra-cellular gene regulatory network dynamics). The choice of the number of micro steps in the gene network dynamics \( k = 10 \) has been taken based on the heuristic that notwithstanding the
configuration of the input node, 10 micro steps are ‘sufficient’ for the network to reach a state corresponding to a switch to TH1 or TH2. (We estimated that by limiting the microdynamics to \( k = 10 \) steps we commit an error of magnitude 1.35\%. In practice this error is much smaller because not all the configurations are reached by the networks given the input cytokines at the mesoscopic level.)

3 DISCUSSION

It is worth at this point to spend few words about the way we use the terms ‘dose’ and ‘concentration’. When we say ‘allergen concentration’ of, e.g. 2000 \( \mu \text{g/mL} \), we actually mean ‘the dose whose resulting concentration is 2000 \( \mu \text{g} \) in a milliliter of blood after a suitable delay of time’, since our simulation space is always a milliliter of blood.

The allergen administration protocol consists of a first injection at initiation (time zero) and a burst injection at day 40 (Fig. 4).

3.1 Histamine release as a consequence of sensitization and challenging

The simulator reproduces all the hallmarks of an IgE-mediated hypersensitive reaction which, in susceptible individuals, culminates in the production of a high concentration of histamine.

This is shown in Figure 4. After injection of a generic allergen at day 0 (sensitization), the immune system develops an immune response toward the TH2 phenotype (Fig. 4a). No histamine is produced but a high fraction of sensitized MCs is observed as a consequence of the modest production of immunoglobulins of the IgE type (Fig. 4e). After the second allergen’s injection at day 40 (challenge) the TH2 count rises even more, the isotype switch is complete increasing the sensitization of MCs (Fig. 4f) and consequently a high level of histamine is produced (Fig. 4d) indicating an hypersensitive response.

3.2 Effects of IFN-\( \gamma \) and IL-4

To further validate our model we study the effects of IFN-\( \gamma \) and IL-4 on the development of IgE or IgG antibodies (Fig. 5). To this end we simulate atopic subjects (\( \beta = 0.15 \)) that get sensitized at day 0 and then are challenged at day 40. At the same time of the challenge we inject a variable amount of IL-4 or IFN-\( \gamma \) and observe the concentration of IgE and IgG produced (peak values).

Results are shown in (Fig. 5 a and b, respectively), where each point is computed averaging the outcome of hundred independent runs. As expected, the level of produced IgE is positively correlated with IL-4 dose, since IL-4 not only favors isotype switch to IgE, but also sustains the switch of TH0 to TH2 cells, further producing IL-4 in a positive feedback (cf. Fig. 1). In contrast, the level of IgE is negatively correlated with the injected IFN-\( \gamma \) dose, since IFN-\( \gamma \) amplifies the effects of IL-12 released by antigen processing cells, and induces TH0 to undergo a class switch to TH1. In addition, IFN-\( \gamma \) supports isotype switch of B cells to IgG. The inverse results are obtained for the effect of IL-4 and IFN-\( \gamma \) on IgG production. As can be seen in Figure 5 our simulation results are in good agreement with in vitro observations (Del Prete et al., 1988).
On the basis of these results we plan to implement a more detailed gene-level dynamics accounting for a higher number of secreted cytokines, signal transducing molecule and transcription factors. In fact, due to the relative simplicity of the network and the low number of intercellular signaling molecules considered, the present model is not well suited to be used to perform virtual knockout experiments where the inhibition of a single molecule has consequences on the overall dynamics of the immune response. Such foreseeable model would have promising application in the development of anti-allergic drugs or treatments. However, there are a number of practical aspects in the design of a gene network that cannot be ignored (first of all the lack of experimental results uncovering the function of certain molecules).

Without any doubt, though very appealing, this bottom-up modeling approach has revealed to be a little baffling. We can therefore conclude that the development of a multi-scale model as the one we have described here has to proceed incrementally, going by very simple steps, adding few elements at a time and, most important, checking the consistency of the resulting overall dynamics with phenotypic data.

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4 CONCLUSIONS

We have built a multi-level simulator that reproduces the hallmarks of an IgE-mediated hypersensitive reaction. It provides an example of how to combine the mesoscopic-level description of immune cells with the microscopic gene-level dynamics.

![Graph](image_url)

**Fig. 5.** IgE and IgG production as a function of the injected (a) IL-4 and (b) 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. Results are in line with in vitro observations (Del Prete et al., 1988). Interleukin concentrations show averages on hundreds of independent runs. Lines are shown to increase readability. The SFM accounts for about 5–15% (error-bars not shown to have neat plots).

It is interesting to note that for low values of the antagonistic cytokine (i.e. IgG versus IL-4 in Fig. 5a and IgE versus IFN-γ in Fig. 5b) the correlation with the immunoglobulin level is smaller than the concurring one. For higher doses of injected cytokine instead, the correlation is much more noticeable, almost linear. This is also evident from the SD that is higher for smaller values of the cytokine (the standard error of the mean σ/√n, with n the number of values in the sample, ranges between 5% and 15%).

Also, in accordance to Del Prete et al. (1988), the same pattern of correlation between IFN-γ/IL-4 and IgE/IgG is found when we simulate non-atopics, i.e. setting the parameter β = 1/2 (not shown).