Workpackage 4

Link: http://www.iac.rm.cnr.it/~filippo/th1_2.html

Work-package objectives

The overall Work-package objectives are to implement a computer code to simulate the phenomena of the emergence of allergic rhinitis or, more generically, a type I hypersensitivity reaction. Besides the realization of the simulation code, a number of other activities have emerged from that central idea. They are briefly outlined below.

Progress of Work package 4

At the end of the second reporting period, corresponding to the end of the project, WP4 has provided a simulation tool of the generic features of type I hypersensitive response at the cellular level that includes a sub-cellular level description in terms of gene regulatory network of the Th1/2 differentiation. The simulator includes a simple interface to input data to the simulator plus a set of scripts to generate graphical outputs to the screen or on image files.

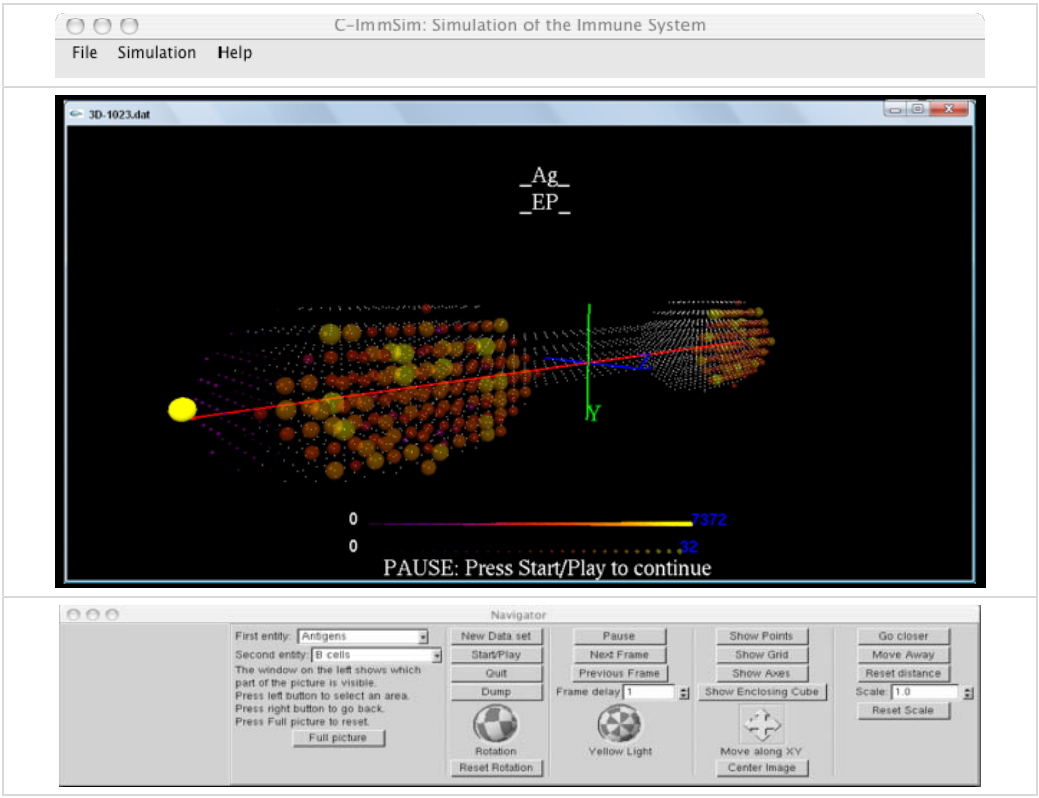

Figure 1 The simulator graphical interface. In the picture the multi-organ simulation is shown.

WP4 member M. Bernaschi has developed most of the graphical user interface shown in Figure 1 and Figure 2. The first of the two graphical modules facilitates input of the parameters of the simulation also providing a consistency check on the values (Figure 1 left panel). A simple interface to gnuplot allows to plot output data as for example distributions or cell population dynamics (Figure 2, right panels). The second graphical module allows the user to display 3D data (this is used only in the MS project described below). The tool visualizes each lattice point of the virtual organ as a dot. Its colour represents the concentration of the agent's population that has been selected by the user by the appropriate lever (Figure 1, bottom).

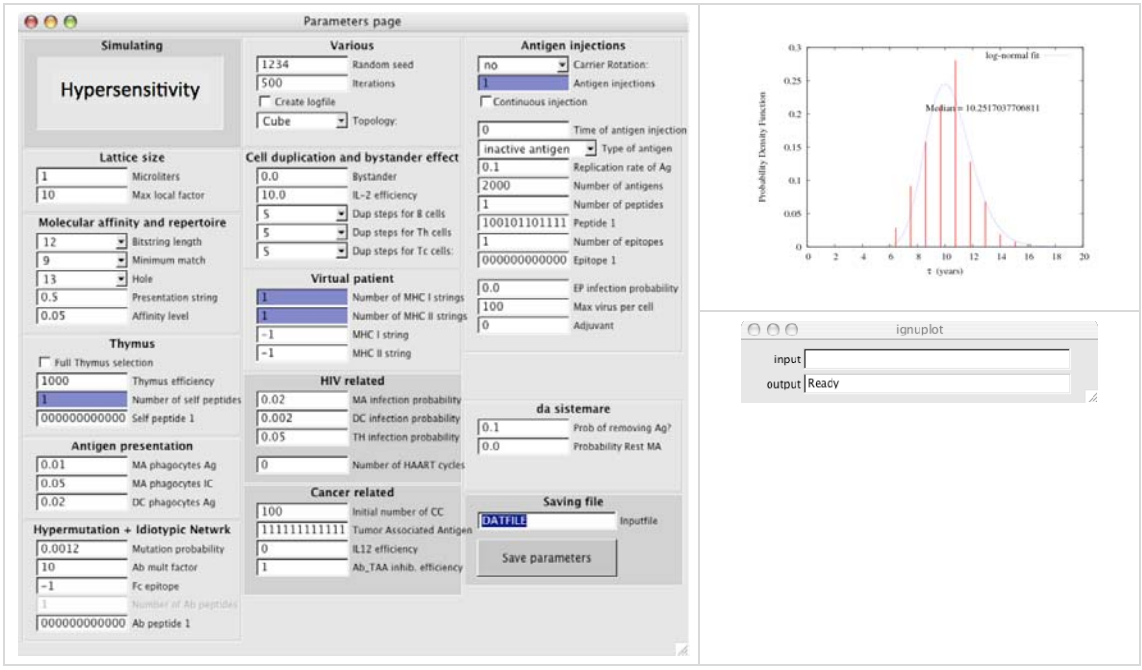

Figure 2 The input control panel (left) and the plotting window providing a simple command-line interface to *gnuplot*.

On a more theoretical side, this study required the identification of a gene level description of a particular process that is pivotal in the onset of the hypersensitivity response. To this purpose WP4 has initially identified the gene network published by Mendoza in 2006 (*Mendoza, L. 2006. A network model for the control of the differentiation process in Th cells.* Bio System *84, 101-114*). This allowed the implementation of the multi-scale simulation tool encompassing gene-regulatory machinery and intercellular activation of the immune actors (see Figure 3).

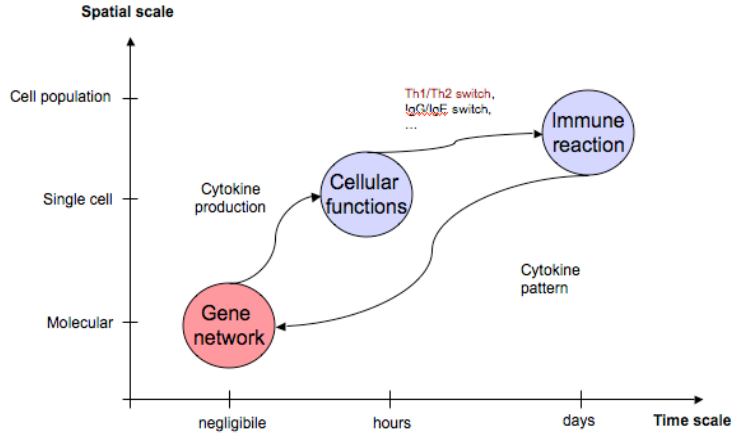

Figure 3 Multi-scale model of type I hypersensitivity reaction achieved by integrating an agent-based model of the immune response with a gene-regulatory dynamics as a Boolean-type dynamical network.

However, a closer look to this network revealed it “too simple” to allow knock out experiment since the outcome of the simulation could minimally be traced down to an accurate analysis of the network itself. In this respect, the simulation, apart from proving that the macroscopic emergent behaviour is correct, does not give any further insight into the allergic disease at the level of gene activation, nor suggests possible treatment based on knocking out this or that gene.

For this reason, as already anticipated in the previous Activity Report (period one activity report), we undertaken a different path: we decided to devise a more detailed gene regulatory

network (GRN) of the Th1/2 using the network of Mendoza as template and employing an hybrid approach based on both text mining and Biogrid protein-protein interactions database retrieval. Then we manually curated the network to include directionality and inhibition/activation information. This provided a GRN to replace the prototypical gene regulatory network used so far with a more realistic and comprehensive one, by taking into account micro-array data and statistical as well as bioinformatics expertise from WP2, WP5, WP6 and WP7. Since this task has required more energy than expected, we focused, at this stage, on this aspect more than on to the simulator itself; the idea being of implementing the network in the simulator at a later stage, once the GRN have proven to deliver what we think. In this respect, it is important to say that the simulator has been purposefully made both modular and expandable, and therefore the implementation of a more detailed GRN other than that those of Mendoza will be possible with a relatively small effort.
The new Th1/2 gene regulatory network is displayed in Figure 4. It has more than 50 genes. Nodes have been classified according whether they are to be considered input, output or internal nodes. This classification facilitates the analysis and is consistent to what we have done in the previous work when we "wired" it in the simulator (see deliverable D13 and publication Santoni et al., 2008).

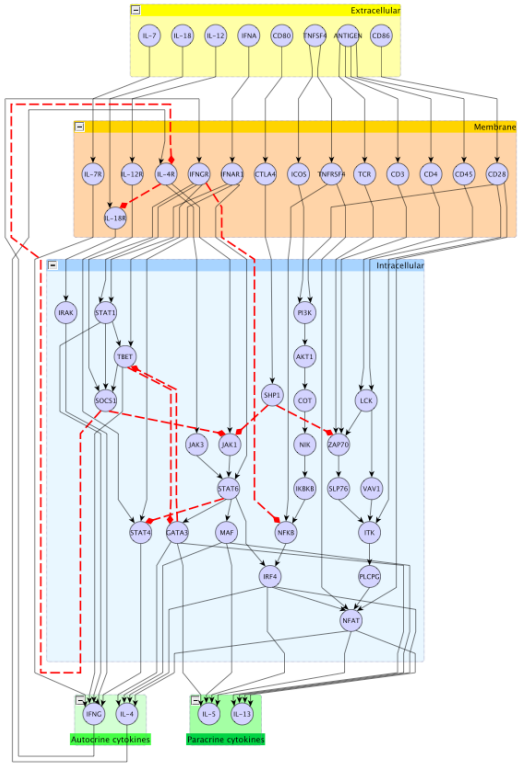

Figure 4 Gene regulatory network of Th1/2 differentiation. Similarly to what we have done in Santoni et al. 2009, nodes have been classified according whether they are to be considered input, output or internal nodes. Black arrows between genes indicate activation, red arrows inhibition.

Recent studies have implicated a large number of genes in Th2 differentiation and also that there is no counter-regulation, as early studies suggested, between genes belonging to the two different phenotypes. The counter-regulatory nature of the Th1 and Th2 differentiation is also supported by some modeling studies. In particular, in a work in progress we aims to identify, study and analyse the gene regulatory network made of the most "significant" genes involved in the Th1/2 differentiation process and to study the combined effects of all the genes by means of computer simulations of the corresponding Boolean gene network to deduce the dynamical properties.

We found that simulation data is compatible with recent biological findings. In particular, the counter-regulatory role of Th1 versus Th2's genes has been challenged by experimental studies during the last few years. For example partner UGOT has actually found increased activity of Th1 in allergen-challenged T cells. Others have made similar observations. This is compatible with recent data and shows the value of modelling to examine combined effects. Additional Experimental studies can take from 3-6 months and involve 2-3 people, and may still not get expected results.

Always during the second reporting period we have brought forward a number of collateral activities. In one of this, the computational model has been used to perform studies of desensitization protocols. The aim of this side-study made in collaboration with partner AOPDIT (WP6) is to use the simulator to estimate the effectiveness of a generic immunotherapy used to desensitize a patient suffering from a form of type I hypersensitivity. In allergic patients, the immunotherapy protocol follows a specific pattern of allergen administration from low to high dosage (Figure 5). The study required a large number of simulations to be performed to assess whether the switch to the Th2 phenotype could be reverted to the non-pathological (allergy-wise speaking) Th1. Unfortunately the first set of runs did not point to definitive conclusions and further study is necessary to understand whether the model need to account for further mechanisms not previously included, that could turn the immune response toward the Th1 phenotype type of response.

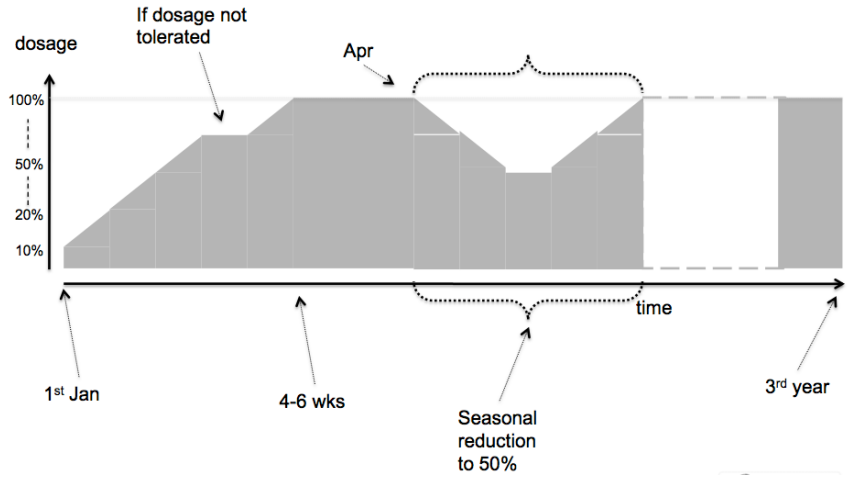

Figure 5 A generic desensitizing immunotherapy protocol for patients suffering from a form of type I hypersensitivity. It follows a specific pattern of allergen administration from low to high dosage. The protocol cannot exclude momentary interruptions of dosage-increase in case of intolerances or dose-reduction during springtime because of the high amount of allergen in the air, to avoid the risk of anaphylactic shocks.

Another parallel activity initiated in this reporting period by collaborating with UNAV (WP8), is to develop a model to simulate the cellular interactions making up the inflammation of brain tissue at the onset of the Multiple Sclerosis (MS). This work in progress required the modification of the simulation code to include specific immunological knowledge related to MS but most importantly, to construct the model as a two compartment models, a lymph node and a portion of the brain. Preliminary results have been presented at the Ninth International Conference on Intelligent Systems Design and Applications (F. Castiglione and Pappalardo F. "Toward multi-organs simulations of immune-pathogen interactions", in Ninth International Conference on Intelligent Systems Design and Applications, ISDA 2009, Pisa, Italy, Nov 30 - Dec 2, 2009, pg. 330-334, 2009. IEEE Computer Society Washington, DC, USA. ISBN: 978-0-7695-3872-3. doi: 10.1109/ISDA.2009.119).

Finally, in collaboration with RR-HF (WP2) and UGOT (WP5) we performed a global analysis of the immune-interactome landscape and inter-complex network in the context of cancer progression. The role of WP4 was to provide algorithmic solutions and calculations of statistical measures on large graphs extracted by manual annotation and high-throughput efforts to catalogue immune genes based on text mining and information theory.

These last two studies are at an advanced stage and manuscripts will be submitted to medical bioinformatics journals quite soon.

Deviations from project work programme and corrective actions

The study of the large network for the Th1/2 switch is currently a work in progress, although at an advanced state. Therefore its implementation in the agent-based simulation tool will certainly go beyond the end of the reporting period.

During the first reporting period WP4 has spent 39 man months of the total 54 declared creating an unbalance of the efforts in the two periods. However the work performed has been exploited and brought forward with much more effort during the second period. The results are satisfying as can be deduced from the following paragraphs on Deliverables and publication activity.

The two postdocs in charge in the first reporting period (i.e., Guido Dell'Acqua and Daniele Santoni) have unexpectedly terminated their appointment at the beginning of the second reporting period (respectively after two months and after 6 months, totalizing about eight months altogether). This fact, together with the difficulties of hiring and training new postdocs, have left us with the only choice of bringing the work forward by ourselves (i.e., using man/months of the CNR personnel).

The overall activity has increased as different lines of investigation have been pursued. The end result amounts in 10 man/months more that what initially planned as indicate in the person-month-status-table.

Deliverables

D12. The simulator it self. This deliverable consists in the computer model relative to the agent-based simulation tool for the allergic reaction to a generic allergen. This deliverable consists in the computer model relative to the agent-based simulation tool for the allergic reaction to a generic allergen.

The code is available under request to f.castiglione@iac.cnr.it.

D14. Visualization tools and various statistical utilities. The simulator D12 is equipped with two graphical modules.

The first of the two facilitates input of the parameters of the simulation also providing a consistency check on the values (Figure 2 left-panel of the section relative to WP4 of the activity report). A simple interface to gnuplot allows plotting output data as for example distributions or cell population dynamics (Figure 2, right-panels of the section relative to WP4 of the activity report).

To this purpose a set of scripts to generate graphical outputs to the screen or on image files have been provided.

The second graphical module allows the user to display 3D data (this is used only in the Multiple Sclerosis project described in the activity report). The tool visualizes each lattice

point of the virtual organ as a dot. Its colour represents the concentration of the agent's population that has been selected by the user by the appropriate lever

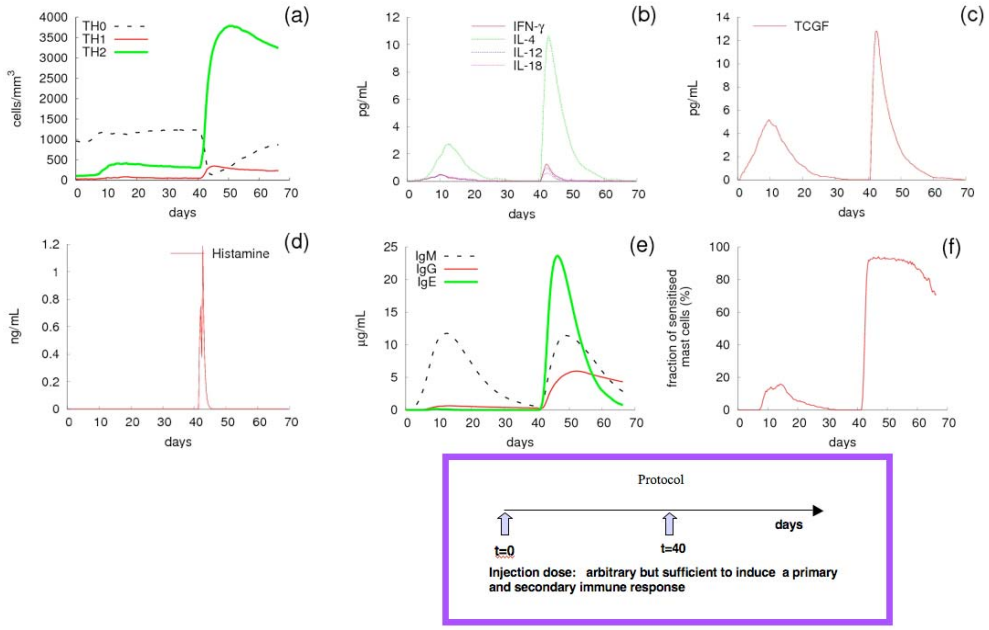

Figure 6. The dynamics of the resulting multi-scale system is consistent with the overall population dynamics of lymphocytes, chemokines and TH1/2 count's ratio during a type I hypersensitivity reaction.

In the second reporting period other publications related to collateral activities are:

- G. Dell'Acqua and F. Castiglione. Stability and phase transitions in a mathematical model of Duchenne muscular dystrophy. *J Theor Biol.* **260(2)**: 283-9 (2009). doi:10.1016/j.jtbi.2009.05.037;
- F. Pappalardo, M. Pennisi, F. Castiglione, S. Motta. Vaccine protocols optimization: in silico experiences. *Biotechnology Advances.* **28**: 82–93 (2010). doi:10.1016/j.biotechadv.2009.10.001;
- F. Castiglione and Pappalardo F. "Toward multi-organs simulations of immune-pathogen interactions", in Ninth International Conference on Intelligent Systems Design and Applications, ISDA 2009, Pisa, Italy, Nov 30 -- Dec 2, 2009, pg. 330--334, 2009. IEEE Computer Society Washington, DC, USA. ISBN: 978-0-7695-3872-3.
- G. Dell'Acqua and F. Castiglione. "A mathematical model of Duchenne muscular dystrophy", in Applied and Industrial Mathematics in Italy III (AIMI III), Proceedings of the SIMAI 9th Congress, Rome, September 15-19, 2008. World Scientific Press (2009).

Two other publications are currently in press or under review:

- F. Castiglione, D. Santoni and M. Pedicini. Implementing agent's rules with gene regulatory networks in mesoscopic-level models of cellular interactions. To appear as book chapter in "A practical guide to bioinformatics analysis", iConcept Press (2010)
- F. Castiglione, D. Santoni, N. Rapin, M. Bernaschi. CTLs' repertoire shaping in the thymus: a Montecarlo simulation. Submitted to *Autoimmunity* (2010)

Milestones

With respect to the planned activity and milestone M10, the various programmed tasks have been accomplished: Analysis of the problem, selection of the most important features of the simulator, definition of modelling architecture, computer programming and code optimization.

Postdocs

During the second period (1.9.2008 – 28.2.2010) the two postdocs hired in the first period (i.e., Daniele Santoni and Guido dell'Acqua) have left the project (respectively in February 2009 and October 2008). Therefore they covered about eight months altogether in the second reporting period.

Collaborations

With respect to the consortium integration issue WP4 has participated to all ComplexDis meetings of the second period (in Rome in March 2009 and in Oslo in October 2009). WP4 has organized and hosted the meeting in Rome in the period March 4-6, 2009.

Filippo Castiglione has visited partner UNAV (WP8) in Barcelona in the period June 30 – July 5, 2009, to start collaborating around the theme of the simulation of Multiple Sclerosis.
Moreover, Marco Pedicini has visited Göteborg (partner UGOT, WP5) in February 2010 to finalize the work on the Th1/2 GRN.

Travels

Travel and other related expenses relative to collaboration activities and project meetings described above are summarized here.

- D. Santoni, Summer School On High Performance Computing, Rome 2008.
- F. Castiglione – Dissemination activity, University of Lyon, Lyon 2009.
- M. Pedicini – Dissemination activity, Inst. de Mathématique de Luminy, Marseille 2009.
- F. Castiglione, D. Santoni, M. Pedicini – ComplexDis Meeting, Roma 2009.
- F. Castiglione – Meeting with partner UNAV (WP8), Barcelona July 2009.
- F. Castiglione, D. Santoni, M. Pedicini – ComplexDis Meeting, Oslo 2009.
- M. Pedicini – Meeting with partner UGOT (WP5), Göteborg 2010.

Simulation of the gene regulatory network for TH1/2 differentiation in an agent-based model of hyper-sensitivity reactions

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 was to integrate a gene regulatory network (GRN) of the TH differentiation in an agent-based model of the hyper-sensitivity 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.

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.

A list of softwares can be found at the link above