BIOINFORMATICS ORIGINAL PAPER Vol. 24 no. 15 2008, pages 1715–1721
doi:10.1093/bioinformatics/btn306

Systems biology

Modeling immune system control of atherogenesis

Francesco Pappalardo[1,2,*], Salvatore Musumeci[3] and Santo Motta[2]

[1]Faculty of Pharmacy, [2]Department of Mathematics and Computer Science, University of Catania, Catania, Italy and [3]Department of Pharmacology, Gynecology and Obstetrics, Pediatrics, University of Sassari and Institute of Biomolecular Chemistry, National Research Council (CNR), Li Punti (SS), Italy

Received on February 25, 2008; revised on May 8, 2008; accepted on June 10, 2008

Advance Access publication June 12, 2008

Associate Editor: Thomas Lengauer

ABSTRACT

Motivation: Atherosclerosis is a disease that is present in almost all humans, typically beginning in early adolescence. It is a human disease broadly investigated, that is amenable to quantitative analysis. Oxidized low-density lipoproteins (LDLs) and their autoantibodies are involved in the development of atherosclerosis in animal models, but their role in humans is still not clear. Computer models may represent a virtual environment to perform experiments not possible in human volunteers that can provide a useful instrument for monitoring both the evolution of atherosclerotic lesions and to quantify the efficacy of treatments, including vaccines, oriented to reduce the LDLs and their oxidized fraction.

Results: We report the application of an agent-based model to model both the immune response to atherogenesis and the atheromatous plaque progression in a generic artery wall. The level of oxidized LDLs, the immune humoral response with production of autoantibodies, the macrophages activity and the formation of foam cells are in good agreement with available clinical data, including the formation of atheromatous plaques in patients affected by hypercholesterolemia.

Availability: The model is available at http://www.immunogrid.eu/atherogenesis/

Contact: francesco@dmi.unict.it

1 INTRODUCTION

Atherosclerosis is a disease affecting arterial blood vessels. It can be considered a chronic inflammatory response in the walls of arteries, in large part due to the deposition of low-density lipoproteins (LDLs), i.e. plasma proteins carrying cholesterol and triglycerides, that determine the formation of multiple plaques within the arteries (Ross, 1999). The formation of these plaques in the artery can lead to a number of cardiovascular (heart and blood vessel) problems reducing both the internal diameter of vessels and the blood flux (Vinereanu, 2006). The exact cause of atherosclerosis is not known, but it is noted that people with certain risk factors are much more likely to develop atherosclerosis than people without those risk factors. Some of these risk factors are beyond a person's control (smoking, obesity), other individuals seem to be genetically more inclined to develop atherosclerosis (familial hypercholesterolemia, diabetes, hypertension) (Romero-Corral *et al.*, 2006). Common denominator in all the form of atherosclerosis is the elevated level of LDL, which is subject to oxidation becoming ox-LDL, that promotes an inflammatory response and immune activation in the artery walls (Berliner and Heinecke, 1996). Early studies demonstrated that ox-LDL can induce activation of monocytes/macrophages, endothelial cells and T cells. The role of macrophage cells in the pathogenesis of atherosclerosis has been repeatedly demonstrated and recently an enzyme (chitotriosidase) produced by activated macrophage cells has been investigated. Plasma chitotriosidase activity has been associated with both the extension and prognosis of atherosclerotic vascular lesions in humans (Artieda *et al.*, 2003, 2007) and its phagocyte-specific expression supports a relevant role in innate immunity (Binder *et al.*, 2002) conditioning the evolution of atherosclerotic lesions. The ox-LDLs engulfed by macrophages form the so called foam cells (Steinberg, 1997). These cells represent the nucleus of the plaques formation. The ox-LDL promotes also immune activation of B cells inducing the production of specific anti-ox-LDL antibody. The role of these antibodies against ox-LDL (OLAB) has been debated (Shaw *et al.*, 2001; Shoji *et al.*, 2000). Originally it was reported that such antibodies could be associated to increased risk of atherosclerosis progression, but the difficulties in their determination did not support the consideration that OLAB could represent another risk factor for coronary vascular disease (CVD) (Brizzi *et al.*, 2002; Orem *et al.*, 2002). In contrast, others demonstrated that OLAB were decreased in patients with early signs of CVD-risk as in borderline hypertension and suggested that OLAB was instead a protection factor (Tinahones *et al.*, 2002, 2005). Persistent and high level of LDLs promote the formation of atheromatous plaques. Pathologically three distinct components are present in the plaques: the atheroma is the nodular accumulation of a soft, flaky, yellowish material at the center of large plaques, composed of macrophages nearest the lumen of the artery; the underlying areas of cholesterol crystals; the calcification at the outer base of older/more advanced lesions, occurring within the deepest and oldest layers of the sclerosed vessel wall.

Atherosclerosis and their anatomical consequences cause two main problems. First, the atheromatous plaques, though long compensated by artery enlargement, eventually lead to plaque ruptures and stenosis (narrowing) of the artery and, therefore, an insufficient blood supply to the organ it feeds. Alternatively, if the alteration artery wall is excessive, then a net aneurysm results. These complications are chronic, slowly progressing and cumulative indicating the progression of disease. Most commonly, soft plaque

*To whom correspondence should be addressed.

suddenly ruptures, causing the formation of a thrombus that will rapidly slow or stop blood flow, leading to death of the tissues fed by the artery. This catastrophic event is called infarction and is not predictable. One of the most common recognized scenarios is thrombosis of the coronary artery causing infarction (a heart attack). Since atherosclerosis is a body wide process, similar events also occur in the arteries of the brain (stroke attack), intestines, kidneys, etc. The severity of events associated to atherosclerosis, often cause of dead or serious invalidate disease, require the realization of prevention treatments. Vaccine research for atherosclerosis is a hot pharmaceutical topic.

We present a model based on the agent-based model (ABM) paradigm (Lollini *et al.*, 2006; Motta *et al.*, 2005; Pappalardo *et al.*, 2005; Thorne *et al.*, 2007) which reproduces clinical and laboratory parameters associated to atherogenesis. The model and its computer implementation (SimAthero simulator) considers all the relevant variables that play an important role in atherogenesis and its induced immune response, i.e. LDL, ox-LDL, OLAB, chitotriosidase and the foam cells generated in the artery wall.

2 SYSTEM AND METHODS

2.1 The biological scenario

Exogenous and endogenous factors induce in humans a very small, first oxidative process of blood circulating native LDLs (minimally modified LDLs or mm-LDLs). In endothelium mm-LDLs are extensively oxidized from intracellular oxidative products and then recognized by the macrophage scavenger receptor. High level and persistent in time LDLs lead to macrophages engulfment and their transformation in foam cells. Contrary, low level of LDLs and their oxidized fraction, lead to the internalization of the oxidized LDLs and subsequent presentation by major histocompatibility complex class II at the macrophages surface. Recognition of ox-LDL by macrophages and naive B cells, leads, by T-helper lymphocytes cooperation, to the activation of humoral response and production of OLAB. When the OLAB/ox-LDL immune complexes are generated in the vascular wall, the macrophages catch them by the Fc receptor or via phagocytosis and destroy ox-LDL in the lysosome system. During this process, the activated macrophage releases chitotriosidase enzyme, that is then used as a marker of macrophage activation.

2.2 The conceptual model

To describe the above scenario one needs to include all the crucial entities (cells, molecules, adjuvants, cytokines, interactions) that biologists and medical doctors recognize as relevant in the game. Using the experience of one of us and the immunological expertise of an immunologist (P.-L.Lollini, personal communication), we summarize entities and interactions that are relevant in modeling the atherogenesis and the elicited immune system response. The model is conceptually showed in Figure 1. We considered both cellular and molecular entities. Cellular entities can take up a state from a certain set of suitable states and their dynamics is realized by means of state changes. A state change takes place when a cell interacts with another cell or with a molecule or both of them. We considered the relevant lymphocytes that play a role in the atherogenesis-immune system response, B lymphocytes and helper T lymphocytes. Monocytes are represented as well and we take care of macrophages. Specific entities involved in atherogenesis are present in the model: LDLs, oxidized LDLs, foam cells, autoantibodies antioxidized LDLs and chitotriosidase enzyme. Cytotoxic T lymphocytes are not taken into consideration because they are not involved in the immune response (only humoral response is present during atherogenesis).

For what concerns molecules, the model distinguishes between simple small molecules like interleukins or signaling molecules in general and more

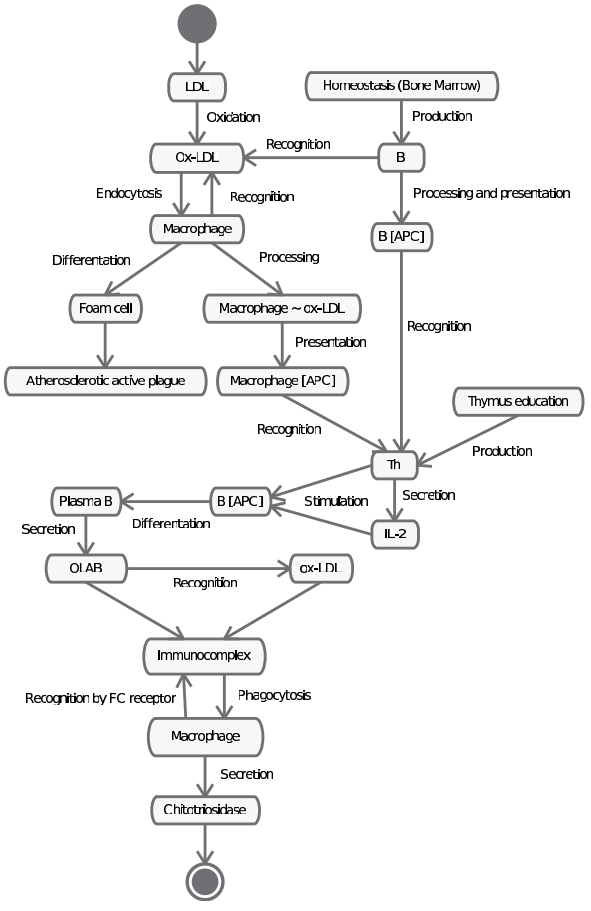

Fig. 1. A global view of the conceptual model.

complex molecules like immunoglobulins and antigens, for which we need to represent the specificity. We only represent interleukin 2 that is necessary for the development of T-cell immunologic memory, one of the unique characteristics of the immune system, which depends upon the expansion of the number and function of antigen-selected T-cell clones. For what is related to the immunoglobulins, we represent only type IgG. This is just because at the actual state we do not need to represent other classes of Ig and because IgG is the most versatile immunoglobulin since it is capable of carrying out all of the functions of immunoglobulins molecules. Moreover IgG is the major immunoglobulin in serum (75% of serum Ig is IgG) and IgG is the major Ig in extra vascular spaces.

Atherosclerosis is a very complex phenomenon which involves also blood dynamics. In this article we are interested only in the immune system processes that control the atherogenesis. These processes may occur in immune system organs like lymph nodes or locally in the artery endothelium. The actual model however does not consider multi-compartments processes and mimics all processes in a virtual region in which all interactions take place. Our physical space is therefore represented by a 2D domain bounded by two opposite rigid walls and left and right periodic boundaries.

Based on our previous experience, we used an ABM technique which allows to describe, in a defined space, the immune system entities with their different biological states and the interactions between different entities. The system evolution in space and in time is generated from the interactions and diffusion of the different entities.

The major advantage of this technique is that the entities and the relationships can be described in terms that are very similar to the biological

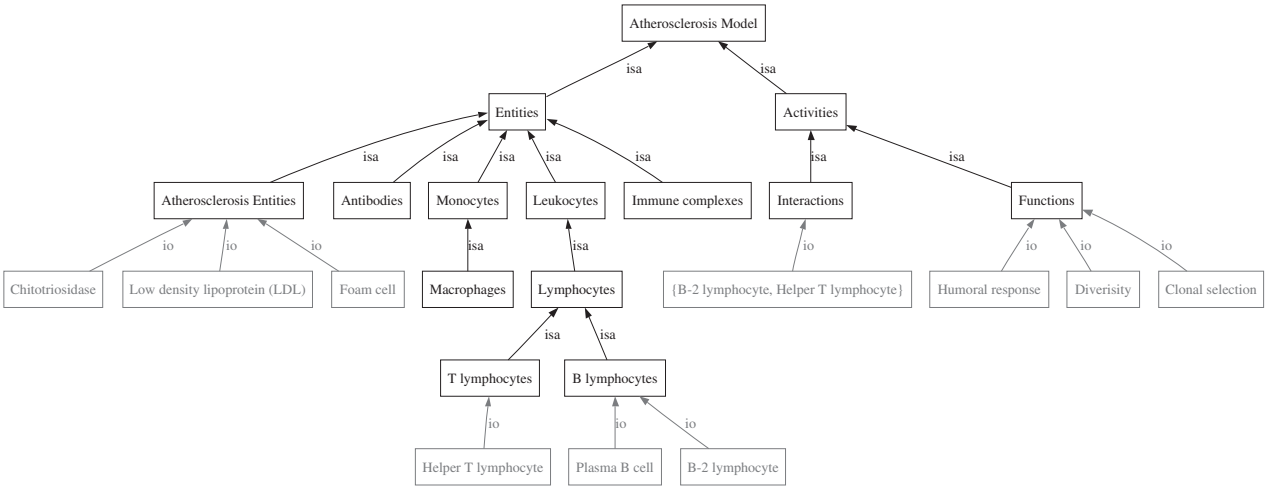

Fig. 2. A global view of the atherogenesis-immune system response model ontology. The figure is not showing all entities and relations present in the ontology (due to limited space available), but only gives a sketch.

world. The intrinsic non-linearity of the system is treated with no additional effort. The approach is thus biologically understandable and relevance is granted by the included biological details; it is flexible and extensible as the behavior of entities is modeled using up-to-date biological knowledge and can be easily modified to reflect observations from biological experiments. Compared to the complexity of the real biological system our model is still very naive and it can be extended in many aspects. However, the model is sufficiently complete to describe the major aspects of the atherogenesis-immune system response phenomenon.

2.3 Model implementation: SimAthero simulator

In designing, so complex, a model, and in particular to eliminate semantic misunderstandings between biologists, medical doctors and computer scientists, it is necessary to list the most important and frequently used concepts coherently defined, to provide an exact semantic specification of the concepts used in an existing schema and to manage and annotate existing database entries consistently. The use of an ontology is the best way to describe the biological entities and concepts. An ontology describes basic concepts in a domain and defines relations among them. We defined an ontology[1] for our ABM, showed in Figure 2. This effort provides a solid infrastructure that is useful to overcome the semantic ambiguities that can arise when we interact in a multidisciplinary field like in the present case.

The computer implementation of the model (SimAthero hereafter) has two main classes of parameters: the first one refers to values known from standard immunology literature (Abbas *et al*., 2007; Celada and Seiden, 1996; Goldspy *et al*., 2000; Klimov and Nikul'cheva, 1999); the second one collects all the parameters with unknown values which we arbitrarily set to plausible values after performing a series of tests (*tuning phase*). Table 1 details the values of the parameters retrieved from the literature.

LDLs values were fixed in order to simulate different patients both in normolipidic condition and in hypercholesterolemic condition. The same applies to ox-LDLs. Initial foam cells level was set to 0 both in normolipidic patients and in hypercholesterolemic patients because we were interested only in the progression of atherogenesis process. Having foam cells values different from 0 is not relevant in the progression of the atherosclerosis. Immune complexes half life is a highly variable parameter because it depends on their chemical composition. However, this was not an important parameter in the simulation of atherogenesis because it did not affect other simulation interactions. We set it to a reasonable value, from an immunological point of view, of about three days.

Parameter hyper_mut is the per-bit mutation probability for the antibodies. The hypermutation rate of antibodies is taken as the suggested value in Celada and Seiden (1996); plasma_rel controls the quantity of OLAB released by a plasma B cell per time step. The value indicated in Table 1 means 10 ng/ml each 8 h; prob_M_Ag is the probability for a macrophage to phagocyte an antigen; prob_M_IC is the probability for a macrophage to phagocyte an immune complex; B_dup is the number of time steps a B cells creates a copy of itself when duplicating; TH_dup's the number of time steps a TH cells creates a copy of itself when duplicating.

Table 1. Parameters of SimAthero

Symbol	Entity	Initial quantity (per μl)	Half life (in days)
B	B lymphocyte	250	3.3
TH	Helper T lymphocyte	1250	3.3
M	Macrophage	125	3.3
LDL	Low density lipoprotein	N/A	2.5
ox-LDL	Oxidized low density lipoprotein	N/A	2.5
IC	Immune complex	N/A	3.3
Chit	Chitotriosidase	0	1.0
OLAB	Autoantibody	0	23.0
FOAM	Foam cell	0	N/A
P	Plasma B cell	0	3.3
IL-2	Interleukin 2	0	1.6

Parameter	Value
hyper_mut	10^{-4}
plasma_rel	10
prob_M_Ag	5×10^{-4}
prob_M_IC	10^{-1}
B_dup	4
TH_dup	4

[1]We used Protégé software, http://stanford.protege.edu.

Looking at the Figure 2, at the same level of entities we find immune system activities. They include both interactions and functions. Functions refer to the main immune system tasks. In particular SimAthero takes care of the diversity of specific elements, major histocompatibility classes restriction, clonal selection by antigen affinity, thymus education of T cells, antigen processing and presentation (both the cytosolic and endocytic pathways are implemented), cell–cell cooperation, homeostasis of cells created by the bone marrow, hypermutation of antibodies, cellular and humoral response and immune memory.

Our model represents receptors and ligands as bit strings and use a string matching rule to model affinity. This clever idea was introduced by Farmer *et al*. (1986) as a way to perform calculations for determining molecular complementarity and predicting the optimal size of an epitope. From immunology, we know that binding is a threshold effect consisting of two components: the affinity of a single receptor and ligand, and the total binding, or avidity of multiple binding pairs. Binding is modeled by a string matching rule by counting the number of positions in the string at which the symbols are complementary (known as Hamming distance). Repertoires are represented in the model as sets of strings. This fundamental modeling abstraction ignores nearly all of the physical and chemical details that determine receptor/ligand interactions. By adopting bit strings, many binding events can be simulated quickly, making it feasible to study large-scale properties of the immune system. Although character strings are a poor representation of the reality, they produced accurate models when benchmarked to experiment, suggesting that the abstraction captures important features of receptor/ligand binding.

In particular, specificity is implemented in SimAthero by a *bit-string polyclonal lattice method*. Bit-string refers to the way the molecules and the specificity among molecules is represented, polyclonal indicates that more clones of different specificity of lymphocytes are represented and lattice means that we use a discrete lattice to represent the space, that is, the space is discrete. The set of lymphocytes receptors is represented by bit-strings of length h which then forms the so called shape space. A clonal set of cells is characterized by the same clonotypic receptor, i.e. by the same bit-string of length l. The potential repertoire of receptors scales as 2^l. The receptor–coreceptor binding among the entities are described in terms of matching between binary strings with fixed directional reading frame. Bit-strings represent the generic binding site between cells (through their receptors) and target molecules (through peptides and epitopes).

Taking into account that a simulator time step is 8 h, we can say that entities in a site are those entities that a single entity encounters during 8 h. An interaction between two entities is a complex action which eventually end with a state change of one or both entities. Specific interactions need a recognition phase between the two entities; recognition is based on Hamming distance and affinity function and is eventually enhanced by adjuvants. When two entities, which may interact, lie in the same lattice site then they interact with a probabilistic law. All entities which may interact and are in the same site have a positive interaction.

The simulator takes care of the main interactions that happens during an immune response against atherogenesis. Interactions included in the model are the following.

1. B lymphocyte recognition of an oxidized LDP antigen. If a B lymphocyte expresses at the cell surface a membrane immunoglobulin which is specific for the antigen, B lymphocyte internalizes the antigen complexed with membrane immunoglobulin and processes into peptides which are then presented by major histocompatibility complex class II at the B lymphocyte surface. Lymphocyte B becomes antigen presenting cell.
2. B lymphocyte and helper T-lymphocyte interaction. If the T receptor (CD4) at the surface of a T-helper lymphocyte binds specifically peptide/major histocompatibility complex class II at the surface of the antigen presenting B-lymphocyte, helper T lymphocyte proliferates and secretes interleukin 2. At the same time, B lymphocyte proliferates and differentiates into a plasma cell.
3. Macrophage and helper T-lymphocyte interaction. If a T-cell receptor (CD4) at the surface of T-helper lymphocyte binds specifically peptide/major histocompatibility complex class II at the surface of antigen processing macrophage cell, helper T lymphocyte proliferates and secretes interleukin 2.
4. Macrophage and immune complex interaction. If a macrophage encounters an immune complex (oxidized LDP autoantibody + oxidized LDP), the macrophage phagocytates the immune complex and secretes chitotriosidase.
5. Macrophage with oxidized LDP. If a macrophage encounters an oxidized LDP, the macrophage internalizes the oxidized LDP and processes it into peptides which are then presented by major histocompatibility complex class II at the macrophage surface. Macrophage becomes antigen presenting cell.
6. ox-LDL with OLAB interaction. If a soluble immunoglobulin specific for the oxidized LDP encounters this antigen, it binds to it and forms immune complex.

Table 2. Tuning parameters of SimAthero

Parameter	Value
nbit_str	12
min_match	8
affinity_level	5×10^{-2}
max_lfact	5
IL2_eff	100%
thym_eff	99.9%
oxidation_rate	1.2%
foam_trans	4

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, so that there is no correlation between entities residing on different sites at a fixed time. The simulation space is represented as a $L \times L$ hexagonal (or triangular) lattice (six neighbors), with periodic boundary conditions to the left and right side, while the top and bottom are represented by rigid walls. All entities are allowed to move with uniform probability between neighboring lattices in the grid with equal diffusion coefficient. In the present release of the simulator chemotaxis is not implemented.

Tuning the model Our model like any other model, has a set of free parameters that can be used to tune the model results with experimental data. The list of these parameters and their final used values are quoted in Table 2. Once the known biological parameters (Table 1) have been fixed, there are many strategies that can be applied in order to determine the values of tuning parameters. We used heuristics and we were lucky to find correct values after some tests that provide results in a reasonable agreement when compared to experimental data in Brizzi *et al*. (2004). The first parameter we set is nbit_str that determines the repertoire size. It indicates the number of bits used to represent the molecules and the cells binding sites like cell receptors and antigen peptides and epitopes. It was set to 12 corresponding to a potential repertoire of $2^{12}=4096$ cell receptors. This is obviously very poor with respect to the real immunological repertoire, but it was sufficient to capture the global behavior of the atherogenesis process. The parameter min_match specifies the minimal number of matching bits that are required to have a non-zero probability to bind; affinity_level is the probability to interact between two binding sites whose match is min_match; max_lfact regulates the probability for a cell that is duplicating to create a new cell; IL2_eff is a factor expressing the efficiency of interleukin 2 in stimulating growth of the lymphocytes; thymus_eff represents the efficiency of the thymus in selecting non-self-reactive thymocytes. In general the fraction of circulating autoreactive TH cells should be below 0.1%; oxidation_rate is the rate of the LDL oxidation. In absence of known risk factors (smoke,

alcohol, diabetes and so on) this rate is about 1.2% of the total LDL (G.Tonolo, personal communication). foam_trans is the threshold after that a macrophage differentiated into a foam cell.

It is worth mentioning that the model is robust in the sense that if the biological parameters are set to reasonable values, the model gives reasonable output. This means, for example, that if we slightly vary parameters such as the initial leukocyte formula, the half life of entities, and so on, the model consistently varies its results, without biological discrepancy when compared with available *in vivo* experimental data.

The model reasonably reproduces experimental data, so it is a descriptive model. However the descriptive properties arise from basic immunological rules of the described processes and not from the specific data we analyze. These rules may be changed to take into account specific pathologies, like familiar hypercholesterolemia, to perform model predictions. Comparison of model predictions with available experimental data will determine the predictive behavior of the model and, eventually requires a cycle of model refinement. We will analyze this point when further data becomes available.

3 RESULTS

We analyzed two broad classes of clinical conditions: health normal patients and hypercholesterolemic patients. The differences among these two groups depend on the LDL level which is high in the last group predisposing to precocious coronary artery disease (CAD). As the risk of generating foam cells and consequently vascular damage appears after at least 2 years of high level of LDL, the computer follow-up of the model was settled to a similar period. Considering that normal subjects maintain their LDL level from 800 ng/microL to 1200 ng/microL, while hypercholesterolemic patients keep it from 1300 ng/microL to 1700 ng/microL we simulated 100 virtual patients for each class varying the correspondent LDL level in the range above mentioned.

Moreover it must be considered that the patients (normolipidics and hypercholesterolemics) differ from their initial immune system repertoire which conditioned the OLAB response. We remind here that both specific and non-specific interactions are stochastically determined using a probability function, which depends upon different parameters computed via random number of generators. We simulated biological diversity changing the seed of the random number of generator for each simulated patient. This yields both a different sequence of probabilistic events and a different initial immune system repertoire.

Figure 3 shows the simulation of 100 virtual patients with level of LDL considered normal. The production of foam cells was considered by the simulator absolutely low (only one patient generated one foam cell per microL). Humoral immune response to the ox-LDL (B) shows a fluctuating behavior of anti-ox-LDL antibodies (mU/ml) where in the first months reaches a maximum of 2240 mU/ml at 20 months. The formation of immune complexes ox-LDL/OLAB stimulate the macrophage cells through the Fc receptor and induce the production of chitotriosidase (D). It is evident that peaks of OLAB correspond to significant reduction of ox-LDL (C) by an active removal mechanism leaded by macrophages. This observation suggests that in normal condition an active mechanism is operating through the generation of anti-ox-LDL in controlling the LDL oxidation and consequently the generation of foam cells. This is in reasonable agreement with human observation reported in Brizzi *et al*. (2004). In fact we calculated a mean OLAB concentration of 550 mU/ml (range 50–2240 mU/ml) and a median of 715 mU/ml

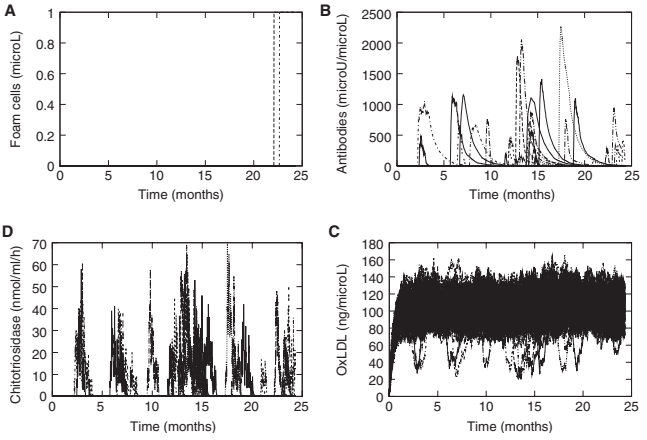

Fig. 3. Simulation results of 100 virtual patients with level of LDL considered normal (from 800 to 1200 ng/microL). The follow-up period is 2 years. The figure shows that foam cells (**A**) formation is practically absent in this class of patients. The oxidized fraction of LDL (**C**) is completely kept under control by the elicited humoral response (**B**) and immune complexes elimination by macrophages activation as indicated by chitotriosidase level (**D**).

whereas Brizzi *et al*. (2004) reported a median of 515 mU/ml (range 88–1549 mU/ml).

Figure 4 shows hypercholesterolemic patients. These patients are distinguished from the normals for high level of LDL, which is associated with high level of oxidation (B) and generation of OLAB (C). The immune response in this condition is insufficient to generate immune complexes in such quantities to induce macrophages in this active removal mechanism. The consequence is that the non-complexed ox-LDLs are absorbed by macrophages and stored inside: the production of foam cells (D) hence is progressively high. Also in this case we obtained results which are in a reasonable agreement with human observation reported in Brizzi *et al*. (2004). In fact we calculated a mean OLAB concentration of 401 mU/ml (range 50–10 120 mU/ml) and a median of 401 mU/ml whereas Brizzi *et al*. (2004) reported a median of 338 mU/ml (range 70–860 mU/ml). For this class of patients we found an aberrant values of OLAB higher than 10 000 mU/ml in just a single case which must not be considered in the final evaluation because it is a spurious data. In fact we performed an extra check changing the random seed of that patient (thus changing both initial immune system repertoire and the sequence of the stochastic interactions) and this aberrant value disappeared. It is worth to mention that 92% of the values were in the range reported in *in vivo* data.

4 DISCUSSION

In this article, we present studies using an ABM to model atherogenesis and its induced immune system response in humans. Very few mathematical models (Cobbold *et al*., 2002; Ibragimov *et al*., 2005) and (to our best knowledge) no computational models of atherogenesis have been developed to date and none, that we are aware off, deal directly both with the role of chitotriosidase marker and the dynamic of atheromatous plaque in subjects with high level of total LDL.

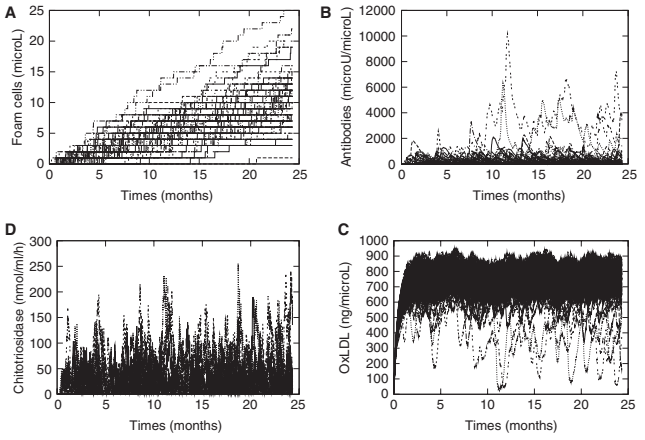

Fig. 4. Simulation results of 100 virtual patients considered hypercholesterolemic (LDL level from 1300 to 1700 ng/microL). The follow-up period is 2 years. The figure shows that foam cells (**A**) formation is appreciable in this class of patients, leading to atheromatous plaques formation and subsequent atherosclerotic lesions. The oxidized fraction of LDL (**C**) is of one order of magnitude greater than the normolipidic patients. This high level of ox-LDL leads to macrophages engulfment and the elicited humoral response (**B, D**) is insufficient to control foam cells formation.

The obtained results confirmed that the initial LDL concentration is determinant in the foam generation as demonstrated by humans observation and in animal models (Ameli *et al.*, 1996). This phenomenon is not clearly understood in humans because many variables could modify the inter-relation among the defined components. The model we reported contributes to simplify the influence of each component in the pathogenesis of atherosclerosis. Moreover the power of this computational model permits for each group of patients to establish a risk factor based on the foam density. The use of computational model allows the operators to monitor the dynamic of atherosclerosis and in future the response to the treatments reducing LDL concentration.

The model was tuned with experimental data collected by Brizzi *et al.* (2003, 2004) where different conditions, normal and hypercholesterolemic diabetic patients were analyzed. In such experimental conditions SimAthero reproduces the same conditions observed in human patients where the relation among ox-LDL and OLAB represents a hypothetical mechanism inducing through the macrophages activation an effective instrument for the clearing of arterial wall. This conclusion has been supported by experimental data obtained in Watanable heritable hyperlipidemic rabbit where the receptor for LDL is genetically missed, and continuous auto-immunization with malondialdehyde-modified LDL resulted in a very high concentration of OLAB against ox-LDL and this intervention of immunization significantly reduce the progression of atherosclerosis (Ameli *et al.*, 1996).

In the present model we did not consider the helper T lymphocytes switching (Th1–Th2). In the evolution of atherosclerotic lesions this switching is relevant because the dynamic of ox-LDL/OLAB is regulated by immune reactivity of individuals. Although Th1 cells may be the major regulators of the lymphocytic influence on the atherogenic process, the cytokine expression of human atherosclerotic lesions suggests that there is a local regulation of Th1 versus Th2 subtypes (Daugherty and Rateri, 2002). Even if we recognize the importance of this effect, we have not enough data to support a model. Future updates of the model will take into account the role of Th1–Th2 switching.

5 CONCLUSION

We presented a model that describes the role of elicited immune response in the atherogenesis. The model applies to the very early stage of the atherosclerosis, i.e. before a calcified plaque is formed. *In silico* experiments on two samples of one hundred virtual humans show reasonable agreements with human observations. The model and its computer implementation is very flexible and new biological entities and interactions can be easily added to the model.

Moreover, the model produced an important suggestion for future biological experiments on the role of OLAB in the activation of the macrophage system to clear the vessels as observed in thalassaemic patients (Brizzi *et al.*, 2002) where the LDL level is low and OLAB concentration is elevated. Actually we are using the model to simulate the behavior of patients with familial hypercholesterolemia, due to the absence of LDL receptor in macrophages which is characterized by an enormous generation of foam cells and severe atherosclerotic lesions. In this condition the model should be useful to control the effect of intensive LDL reducing treatments (i.e. plasmapheresis) and high dosage statine treatments. In this framework if SimAthero simulations predictions will be experimentally validated, it will be possible to obtain precious information on the duration of treatment and their frequency. Results in this way will be published in due course.

ACKNOWLEDGEMENTS

We would like to thank Patrizia Brizzi and Giancarlo Tonolo that produced clinical and laboratory data that were used to tune the model. We also thank Prof. Pier-Luigi Lollini for his valuable help in understanding relevant immunological aspects of the immune system response to atherogenesis.

Funding: This work was supported under the EC contract FP6-2004-IST- 4, No.028069 (ImmunoGrid).

Conflict of Interest: none declared.

REFERENCES

Abbas,A.K. *et al.* (2007) Cellular and molecular immunology. 6th edn. Philadelphia, USA, Saunders.

Ameli,S. *et al.* (1996) Effect of immunization with homologous LDL and oxidized LDL on early atherosclerosis in hypercholesterolemic rabbits. *Thromb. Vasc. Biol.*, **16**, 1074–1079.

Artieda,M. *et al.* (2003) Serum chitotriosidase activity is increased in subjects with atherosclerosis disease. *Arterioscler. Thromb. Vasc. Biol.*, **23**, 1645–1652.

Artieda,M. *et al.* (2007) Serum chitotriosidase activity, a marker of activated macrophages, predicts new cardiovascular events independently of C-Reactive Protein. *Cardiology*, **108**, 297–306.

Berliner,J.A. and Heinecke,J.W. (1996) The role of oxidized lipoproteins in atherogenesis. *Free Radic. Biol. Med.*, **20**, 707–727.

Binder,C.J. *et al.* (2002) Innate and acquired immunity in atherogenesis. *Nat. Med.*, **8**, 1218–1226.

Brizzi,P. *et al.* (2002) Oxidized LDL antibodies (OLAB) in patients with beta-thalassemia major. *J. Atheroscler. Thromb.*, **9**, 139–144.

Brizzi,P. *et al.* (2003) Plasma lipid composition and LDL oxidation. *Clin. Chem. Lab. Med.*, **41**, 56–60.

Brizzi,P. *et al.* (2004) Autoantibodies against oxidized low-density lipoprotein (ox-LDL) and LDL oxidation status. *Clin. Chem. Lab. Med.*, **42**, 164–170.

Celada,F. and Seiden,P.E. (1996) Affinity maturation and hypermutation in a simulation of the humoral immune response. *Eur. J. Immunol.*, **26**, 1350.

Cobbold,C.A. *et al*. (2002) Lipoprotein oxidation and its signicance for atherosclerosis: a mathematical approach. *B. Math. Biol.*, **64**, 65–95.

Daugherty,A. and Rateri,D.L. (2002) T lymphocytes in Atherosclerosis. The Yin-Yang of Th1 and Th2 influence on lesion formation. *Circ. Res.*, **90**, 1039–1040.

Farmer,J.D. *et al*. (1986) The immune system, adaption, and machine learning. *Phisica D*, **22**, 187–204.

Goldsby,R.A. *et al*. (2000) In Austen,K.F. *et al*. (eds) *Kuby Immunology*. W.H. Freeman and Company, New York.

Ibragimov,A.I. *et al*. (2005) A mathematical model of atherogenesis as an inflammatory response. *Math. Med. Biol.*, **22**, 305–333.

Klimov,A.N. and Nikul'cheva,N.G. (1999) *Lipid and Lipoprotein Metabolism and its Disturbances*, Piter Kom, St. Petersburg.

Lollini,P.-L. *et al*. (2006) Discovery of cancer vaccination protocols with a genetic algorithm driving an agent based simulator. *BMC Bioinformatics*, **7**, doi:10.1186/1471-2105-7-352.

Motta,S. *et al*. (2005) Modelling vaccination schedules for a cancer immunoprevention vaccine. *Immunome Res.*, **1**, doi:10.1186/1745-7580-1-5.

Orem,C. *et al*. (2002) The effects of lipid-lowering therapy on low-density lipoprotein auto-antibodies: relationship with low-density lipoprotein oxidation and plasma total antioxidant status. *Coron. Artery Dis.*, **13**, 56–71.

Pappalardo,F. *et al*. (2005) Modeling and simulation of cancer immunoprevention vaccine. *Bioinformatics*, **21**, 2891–2987.

Romero-Corral,A. *et al*. (2006) Update in prevention of atherosclerotic heart disease: management of major cardiovascular risk factors. *Rev. Invest. Clin.*, **58**, 237–244.

Ross,R. (1999) Atherosclerosis–an inflammatory disease. *N. Engl. J. Med.*, **340**, 115–126.

Shaw,P.X. *et al*. (2001) Human-derived anti-oxidized LDL autoantibody blocks uptake of oxidized LDL by macrophages and localizes to atherosclerotic lesions in vivo. *Arterioscler. Thromb. Vasc. Biol.*, **21**, 1333–1339.

Shoji,T. *et al*. (2000) Inverse relationship between circulating oxidized low density lipoprotein (oxLDL) and anti-oxLDL antibody levels in healthy subjects. *Atherosclerosis*, **148**, 171–177.

Steinberg,D. (1997) Low density lipoprotein oxidation and its pathobiological significance. *J. Biol. Chem.*, **272**, 20963–20966.

Thorne,B.C. *et al*. (2007) Combining experiments with multi-cell agent-based modeling to study biological tissue patterning. *Brief. Bioinform.*, **8**, 245–257.

Tinahones,F.J. *et al*. (2002) Increased levels of anti-oxidized low-density lipoprotein antibodies are associated with reduced levels of cholesterol in the general population. *Metabolism*, **51**, 429–431.

Tinahones,F.J. *et al*. (2005) Influence of age and sex on levels of anti-oxidized LDL antibodies and anti-LDL immune complexes in the general population. *J. Lipid Res.*, **46**, 452–457.

Vinereanu,D. (2006) Risk factors for atherosclerotic disease: present and future. *Herz*, **31** (Suppl. 3), 5–24.