AGENT BASED MODELING OF HUMORAL IMMUNE RESPONSE TO ATHEROGENESIS

Francesco Pappalardo[1,2], Francesca Gullo[2], Roberto Catanuto[1], Emilio Mastriani[3], Marzio Pennisi[1], Salvatore Musumeci[4], Santo Motta[1]

(1) Dept. of Mathematics and Computer Science, University of Catania, Italy
{francesco,catanuto,mpennisi,mastriani,motta}@dmi.unict.it

(2) Faculty of Pharmacy, University of Catania, Italy

(3) Consorzio COMETA, Italy

(4) Department of Pharmacology, Gynecology and Obstetrics, Pediatrics, University of Sassari
Institute of Biomolecular Chemistry, National Research Council (CNR), Li Punti (SS), Italy.
smusumeci@dipchi.unict.it

Keywords: Agent based models, atherosclerosis, personalized medicine, system biology, immune system.

Abstract. Atherosclerosis is a pathology where the immune system control plays a relevant role. We present studies on the increased atherosclerosis risk using an agent based model of atherogenesis. It is well known that the major risk in atherosclerosis is the persistent high level of low density lipoprotein (LDL) concentration. However, it is not known if short period of high LDL concentration can cause irreversible damage and if reduction of the LDL concentration (either by life style or drug) can drastically or partially reduce the already acquired risk.

A model approach to this problem by simulating four different clinical situations of the same virtual patient is shown. In the first one the patient lifestyle maintains the concentration of LDL in a no risk range. This is the control case simulation, to be compared with other ones. In the second and in the third simulations, the life style of the virtual patient rises the LDL concentration to a risk level. Countermeasures to reduce the LDL concentration are taken late, in the former case, and early in the latter one. Differences in the foam cells formation can be interpreted as permanent or non permanent risk effects. Finally we consider a virtual patient whose life style rises many time the level of LDL concentration just above the normal but this is quickly reduced using appropriate treatments.

Those preliminary results show that the problem of correct timing of appropriate treatment need to be carefully investigated in order to prevent permanent damages.

1 Introduction

Atherosclerosis, a disease affecting arterial blood vessels, is one of most common disease of the developed countries. It is, 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 [11, 18]. The origin of atherosclerosis is still not fully understood. However there are risk factors which increase the probability of developing atherosclerosis in humans. Some of these risk factors are beyond a person's control (smoking, obesity), others seem to have genetic origin (familial hypercholesterolemia, diabetes, hypertension) [17]. 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 [4]. The formation of atherosclerotic plaques in the artery reduces both the internal diameter of vessels and the blood flux leading to a number of serious pathologies [25]. Early studies

demonstrated that ox-LDL can induce activation of monocytes/macrophages, endothelial cells and T cells. . Ox-LDLs engulfed by macrophages form the so called foam cells [22]. These cells represent the nucleus of the plaques formation. Ox-LDL promotes also immune activation of B cells inducing the production of specific anti ox-LDL antibody (OLAB).

Atherosclerosis and their anatomical consequences cause sever problems. Stenosis (narrowing) and aneurysm of the artery are chronic, slowly progressing and cumulative effects indicating the progression of atherosclerotic disease. In both case the result is an insufficient blood supply to the organ fed by the artery. Most commonly, soft plaque suddenly ruptures, causes 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. The most common event is thrombosis of the coronary artery causing infarction (a heart attack): However, since atherosclerosis is a body wide process, similar events also occur in the arteries of the brain (stroke attack), intestines, kidneys, etc. Those atherosclerosis associated events often cause of dead or serious invalidate diseases and require preventive treatments. Vaccine research for atherosclerosis is a hot pharmaceutical topic.

Recently we proposed a model based on the Agent Based Model (ABM) paradigm [16] 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.

In this paper we show how such a model could be used in preventing the risk of formation of atherosclerotique plaque by adopting the appropriate action to decrease in time the level of LDL concentration in the blood. For this purpose we will analyze four different situation over a time scale of two years. The standard normal patient where no foam cells are formed; a patient who take appropriate treatments as soon as the level of LDL exceeds normal levels; a patient having high level of LDL but who delay to apply appropriate treatments and finally a patient who may have many events of high level of LDL but takes immediately appropriate treatments.

The plan of the paper is the following. In §2 we briefly describe the model of the Immune control of atherogenesis; in §3 we describe our simulated patients and show the simulator results. We briefly draw conclusions and future extension of this work in §4.

2 Description of the model

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 low density lipoproteins 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.

The 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. The model described in [16] contains entities and interactions which both

biologist and MD considered relevant to describe the process. Atherosclerosis is a very complex phenomenon which involves many components some of them not fully understood. In the present version of the simulator we considered 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. To describe the Immune processes 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: low density lipoproteins, oxidized low density lipoproteins, foam cells, auto antibodies anti oxidized low density lipoproteins 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).

Molecular entities The model distinguishes between simple small molecules like interleukins or signaling molecules in general and more 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 just because at the actual state we don't 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.

The actual model 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. This biological knowledge is represented using an ABM technique. This 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. 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.

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 [10, 13] [1, 8]; 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 2 details the values of the parameters retrieved from the literature.

Table 2 **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	Auto-antibody	0	23.0
FOAM	Foam cell	0	N/A
P	Plasma B cell	0	3.3
IL-2	Interleukin 2	0	1.6

The simulator takes care of the main interactions that happens during an immune response against atherogenesis. Interactions included in the model are the following: *(i)* B lymphocyte recognition of an oxidized low density lipoprotein antigen; (ii) B lymphocyte and helper T lymphocyte interaction. *(iii)* Macrophage and helper T lymphocyte interaction. *(iV)* Macrophage and immune complex interaction. *(v)* Macrophage with oxidized low density lipoprotein. *(vi)* ox-LDL with OLAB interaction.

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.

Once the known biological parameters (Table 2) have been fixed, the values of tuning parameters can be found using different strategies. We used a heuristic "trial and error method" and after some tests we found values that provided results in a reasonable agreement when compared to experimental data in [5, 6, 7]. 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, or known risk factors, like smoke, alcohol, diabetes and so on, to perform model predictions.

LDLs values can be fixed in order to simulate different patients both in normolipidic condition and in hypercholesterolemic condition. The same applies to ox-LDLs. However human habits change with time and personal life style. A normolipidic patient can change its attitude becoming an hypercholesterolemic one and vice versa. For this reason we allow the simulator to accept varying life style conditions and preventive actions to decrease risk factors.

3 Results

The model described include the possibility of mimicking biological diversity between patients. The general behavior of a class of virtual patients arise from the results of a suitable set of patients, i.e., the mean values of many runs of the simulator of different patients under the same conditions. The class of virtual patients described by the model were tuned against *human* data data collected by [6, 7] where different conditions, normal and hypercholesterolemic diabetic patients were analyzed. For the aim of the present investigation we consider only a single patient simulation (i.e., we run the simulator only once) and deduce the behavior from the experience of the general case [16].

In this section we analyze the behavior of the same patient in four broad class of clinical conditions to show how SimAthero could be used in order to analyze and predict the effects of various

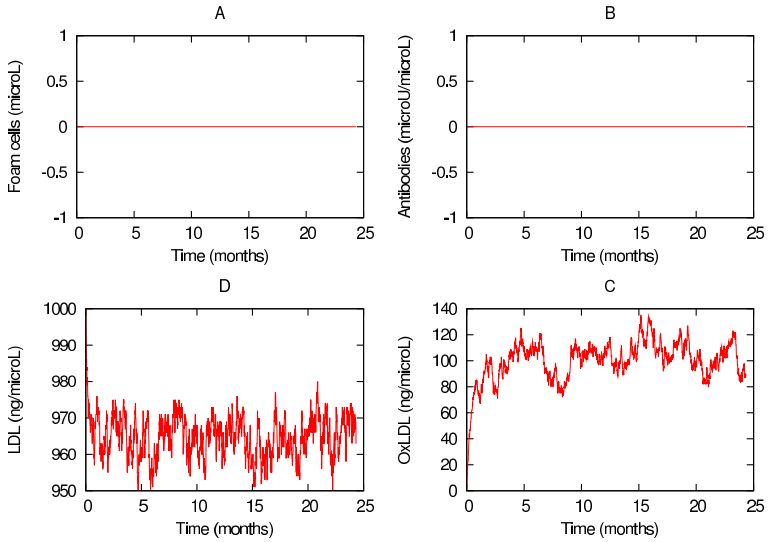

Figure 1: Simulation results of a virtual patient with level of LDL (D) considered normal. The follow-up period is two years. The figure shows that foam cells (A) formation is absent in this patient. The oxidized fraction of LDL (C) is completely kept under control by the patient and no humoral response is present (B).

LDL levels in blood. The normal patient simulation is used as control experiment for the other simulations. The differences among these four clinical conditions depend on the LDL level and the time interval which occurs between the time in which concentration of LDL rise above normal level and the time in which the patient take appropriate treatments (lifestyle o drug) to reduce it to normal level.

A patient with a LDL level of roughly 950-970 ng/μl of blood is considered normal in clinical practice and he has with very low risk of atheroslerotique plaque. The results of SimAthero for a virtual normal patient (Figure 1) show that he will not support the formation of foam cells (panel A) and, as a consequence, the beginning of atherogenesis process is absent. The percentage of the oxidized fraction of LDL (panel C) don't foster humoral response, as it is completely removed by liver in this condition.

We then simulated a scenario in which a patient, due to several reasons (diet, life style, oxidative agents and so on, so forth) leads its LDL level at 1300 ng/μl at day 60, taking it to 1700 ng/mul after ten days. This hypercholesterolemic level is kept to this threshold for about three months. Then, after a blood analysis, the subject starts a pharmacological care at day 160. The care takes about two weeks to take the LDL at normal level (day 174). Looking at figure 2 panel A, one can observe 11 foam cells per μl. This leads to a small atherogenesis process due to the high level of LDL (panel D) for a period of three months. The oxidized fraction of LDL (panel C) leads to a humoral response that, however, is not able to protect the organism against the foam cells formation.

On the contrary if the same virtual patient takes only two weeks to start the pharmacological cure, the damage is negligible. Looking at figure 3 one can observe that the early treatment allows the organism, helped by the humoral response, to limit the damage and the consequent atherogenesis process.

Lastly (figure 4), we analyzed a virtual patient that initially takes its LDL level to small peaks, causing no damage. After that, he takes its LDL level to a hypercholesterolemic behavior, generating a small damage, as shown in panel A. This shows that small LDL alteration are completely taken under control by the normal behavior of the organism, but high LDL peaks lead to foam cells formation and then to the beginning of the atherogenesis process.

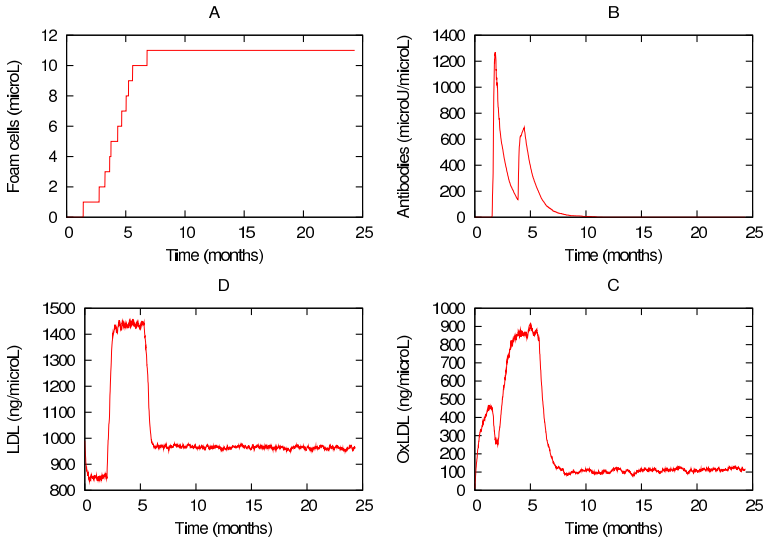

Figure 2: Simulation results of a virtual patient with level of LDL (D) considered at high risk. The follow-up period is two years. The figure shows that foam cells (A) formation is present, leading to an atherogenesis process. The oxidized fraction of LDL (C) is high and the elicited humoral response (B) is not able to limit the foam cells formation.

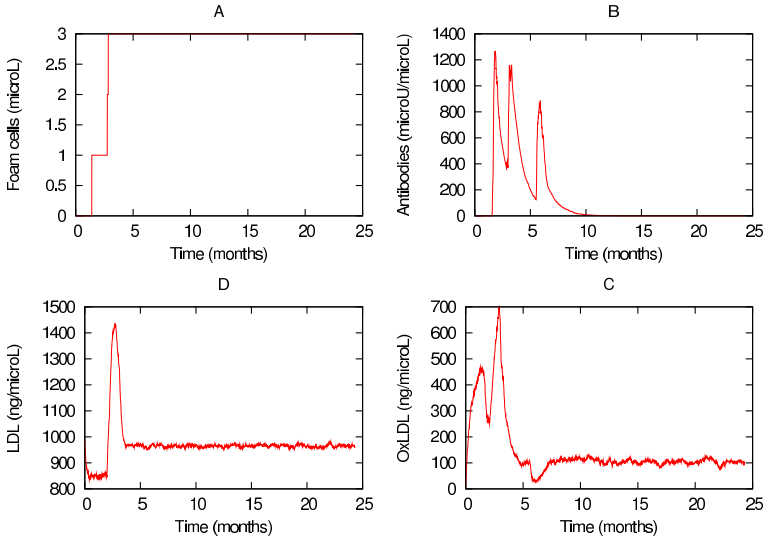

Figure 3: Simulation results of a virtual patient with level of LDL (D) considered at high risk. However the patient was able to take a pharmacological cure against LDL in time. The follow-up period is two years. The figure shows that foam cells (A) formation is negligible. The oxidized fraction of LDL (C) is high but the early cure and the elicited humoral response (B) is able to limit the foam cells formation.

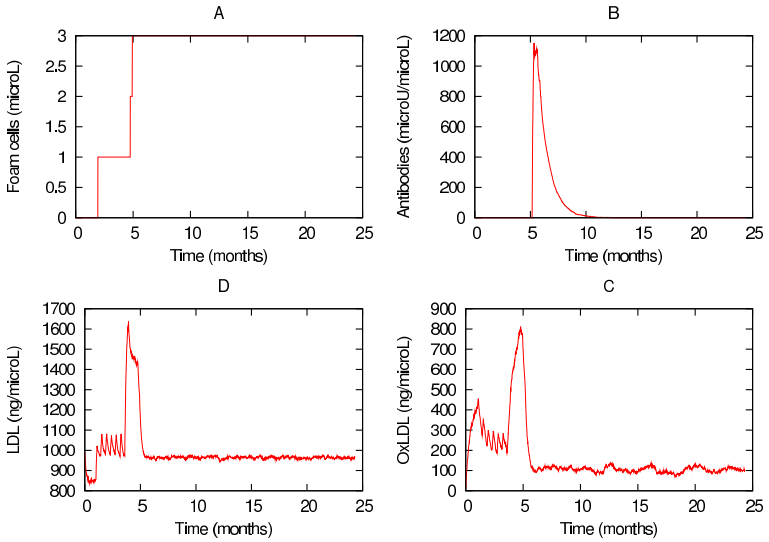

Figure 4: Simulation results of a virtual patient with level of LDL (D) considered quasi-normal at the beginning ant then at high risk. The follow-up period is two years. The figure shows that foam cells (A) formation is negligible in the first time, but becomes important soon after. The oxidized fraction of LDL (C) is high in the second period, and the elicited humoral response (B) is not able to limit the foam cells formation.

4 Conclusions

Atherosclerosis is a pathology where the immune control plays a relevant role. In this article, we presented studies on the increased atherosclerosis risk using an ABM model of atherogenesis and its induced immune system response in humans. Very few mathematical models [9, 12] and (to our best knowledge) no computational models of atherogenesis have been developed to date.

It is well known that the major risk in atherosclerosis is persistent high level of LDL concentration. However it is not known if short period of high LDL concentration can cause irreversible damage and if reduction of the LDL concentration (either by lfe style or drug) can drastically or partially reduce the already acquired risk.

Using an ABM cellular model describing the initial phase of plaque formation (atherogenesis) we are able to simulate the effect of life style which increases the risk of atherosclerosis and the effect of countermeasures represented by changing life style or drugs. This is, to the best of our knowledge, the first analysis of atherosclerosis risk performed using a model.

Compared with the complexity of realistic biology it is very naive but we believe is enough accurate to catch the role of an important component of the process. Results show that in case of high level of risk factor countermeasures must be taken very rapidly to prevent permanent damages.

The interest of modeling atherosclerosis risk is clearly addressed to human. however for the modeling purpose human data may not be the best starting point. We are looking for mouse model which could be used to a detailed description of the immune processes in atherogenesis. Work in this perspective is in progress and results will be published in due course.

Acknowledgments

This work was supported under the EC contract FP6-2004-IST- 4, No.028069 (ImmunoGrid). This work makes use of results produced by the PI2S2 Project managed by the Consorzio COMETA, a project co-funded by the Italian Ministry of University and Research (MIUR) within the Piano Operativo Nazionale ”Ricerca Scientifica, Sviluppo Tecnologico, Alta Formazione” (PON 2000-2006). More information is available at: http://www.pi2s2.it and http://www.consorzio-cometa.it.

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