A discrete/continuous model of anti-HIV response and therapy

Paola Paci, Filippo Castiglione, Massimo Bernaschi  
Istituto Applicazioni Calcolo (IAC) "Mauro Picone"  
National Research Council (CNR)  
00161 Rome, Italy  
[p.paci,f.castiglione,m.bernaschi]@iac.cnr.it

Valentina Baldazzi  
INRIA Rhône-Alpes  
655 avenue de l’Europe  
Montbonnot  
38 334 Saint Ismier Cedex France

Abstract

We present an immune system simulator based on a hybrid discrete/continuous approach. In particular, simulated lymphocytes, antigens and immunocomplexes are treated as stochastic discrete entities whereas interleukins, chemokines and in general small signalling molecules are described as continuous variables ruled by differential equations.

The version of the model we present here is customized to reproduce the immune response to HIV and the effects of an anti-retroviral therapy. Our goal is to suggest new therapeutic strategies that can be tested in a computer.

1. Introduction

In the last decades the field of immunology has provided several challenging cues to the development of theoretical models. The main goal is to deduce macroscopic properties of complex biological systems from the interactions among elementary components. In this framework, discrete models based on concepts like cellular automata have been used to describe a number of biological phenomena including the very complex mechanisms of the immune system response. Further enhancements of such discrete models lead to the Agent Based Modeling (ABM) paradigm in which entities interact each other through a set of deterministic and/or stochastic rules.

One of such models is due to F. Celada and P.E. Seiden (CS-model) that in early nineties attempted to build a general immune system simulator based on stochastic cellular automata [4]. The model incorporated a lot of immunological details although many features of the immune system were described in a oversimplified way. We have been working for a number of years on the development of a simulator that includes most of the features of the original model, but also offers a number of enhancements in terms of performance, flexibility and fidelity to the real immune system. In this paper we describe recent additions/extensions included in the simulator in order to study the infection caused by Human Immunodeficiency Virus (HIV) and we report some interesting results about the efficacy of Highly Active Anti-Retroviral Therapy (HAART) based treatments in keeping under control the growth of the virus. Our aim is to obtain reliable in machina tests of new therapeutic schedules for patients infected by HIV.

The paper is organized as follows: section 2 describes the main features of our simulator with special emphasis on the recent enhancements for the simulation of HIV infection. Section 3 describes how we have simulated the therapy and shows results that are in very good agreement with clinical observations and predictions.

2. Discrete/continuous hybrid approach

The agent based simulator, named C-IMMSIM [1], consists of agents living on a three-dimensional Euclidean lattice.

While in a cellular automata based model, time is discrete and the state of a lattice point at time $t$ is a function of a finite number of lattice points (the neighborhood) at time $t - 1$, in our agent based model, the interactions are local as a large number of entities may sit on each single lattice site (i.e. within the same unit of volume).

Interactions are coded as probabilistic rules that define the entities’ transition from a state to another. Thus, conversely to classic cellular automata models, in C-IMMSIM there is no correlation between entities residing on different sites at a fixed time step and the deterministic character of automata dynamics is replaced by a stochastic behavior. However, at the end of each time step entities diffuse from site to site introducing spatial correlations.

The simulated volume corresponds to a fraction of a lymph node. Lymph nodes are small, ellipsoid structures placed along the vessels of the lymphatic system where disease-fighting white blood cells are produced. The three-dimensional mesh we use recalls the typical ellipsoid shape
of a lymph node, as shown in Fig. 1.

Agents are cells and molecules with specific characteristics (i.e. molecular receptors, half life, etc.). In Fig. 1 some entities belonging to the same volume unit are represented: white blood cells (T helper and B lymphocytes), antibodies (Ab), macrophages (MA) and dendritic cells (DC).

At any time each agent can be in one of a set of possible states (naive, active, resting, duplicating, etc.). Entities change their internal state when they interact with other entities. For this reason the model is also called stochastic stage structured.

Another characteristic of this model is that all molecules and cells' binding sites (e.g. cell receptors) are modeled as binary strings of finite length. Models of this kind are called binary-string models in the computational-immunology field.

Each interaction requires cell entities to be in a specific state. Once this condition is fulfilled, the interaction probability depends on the effective binding between receptors. In particular we define as affinity function a monotonic (i.e. exponential) function of the Hamming distance in the space of the bit-strings.

From the modeling perspective it is worth to stress that the model considers in a different way simple "small" molecules like interleukins or chemokines (i.e. molecular carriers of physiological signals used by cells to acknowledge the occurrence of certain events) and more complex molecules like immunoglobulins. For "small" molecules (i.e. small molecular weight) we only need to take into account the concentration on each lattice point and thus we can use partial differential equations (PDE) to describe their dynamics. In other words, the concentration of interleukins is a continuous quantity that changes in a deterministic way. For other, more complex, molecules that carry specificity we need to use a more complex data structure. Therefore they are represented as individual (hence discrete) agents.

Examples of this kind of entities are immunoglobulins as well as antigens (these could also be simple molecules but in general they are more complex structures like virus or bacteria). From this viewpoint C-IMMSIM can be considered as an "hybrid" model (ABM + PDE) where cells, antigens and antibodies are treated with a stochastic discrete approach (ABM) whereas interleukins are described by using PDEs.

In the original formulation C-IMMSIM described the diffusion of all entities on the lattice at each time step with an equal diffusion coefficient and without any preferential direction, that is, there was no description of the phenomenon of chemotaxis (i.e. the phenomenon in which bodily cells, bacteria, and other single-cell or multicellular organisms direct their movements according to certain chemicals in their environment). Thus cellular and molecular entities moved randomly inside the lattice volume making cells encounters quite inefficient. Actually, lymphocytes mobility plays a central role in determining the efficiency of the immune response. White blood cells continuously recirculate between blood and lymph channels, monitoring and detecting foreign antigens inside the body’s tissues. In particular, within the lymphoid organs, diffusion and chemotaxis affect the cellular organization and the dynamics of interactions among immune cells.

Recently, we added to the simulator a first simplified description of the chemotaxis phenomena. According to that, lymphocytes move following the gradient of their specific chemokines. As for the interleukines, chemokines motion is modeled by a PDE for each particular molecular specie. The equations describing their diffusion is of parabolic type (similar to the heat equation) with the addition of a degradation term that takes into account for the finite half-life of the molecules:

\[
\frac{\partial c}{\partial t} = D \nabla^2 c - \lambda c
\]  

(1)
where \( c = c(x) \) is the concentration of chemokines, \( D \) is the diffusion coefficient and \( \lambda \) is the half-life [14, 7].

In order to guarantee the numerical stability of the above equation we need to use an integration step that satisfies the following relation:

\[
D \left( \frac{dt}{dx^2} + \frac{dt}{dy^2} + \frac{dt}{dz^2} \right) \leq \frac{1}{2} \tag{2}
\]

where \( dx, dy \) and \( dz \) are the integration steps in the three spatial dimensions. In our model \( dx = dy = dz \).

Moreover, for the numerical resolution of Eq. 1 we employ the approximation of derivatives by finite differences. In particular we use a forward difference for the temporal derivative and a central difference for the spatial derivative.

A forward difference is an expression of the form

\[
\frac{\partial c}{\partial t}[f](x) = f(x + h) - f(x). \tag{3}
\]

whereas a central difference is given by

\[
\frac{\partial c}{\partial t}[f](x) = f(x + \frac{1}{2} h) - f(x - \frac{1}{2} h). \tag{4}
\]

Hence, the finite differences divided by \( h \) approximate the first order derivatives when \( h \) is small. In an analogous way one can obtain finite difference approximations to higher order derivatives. For example, by using the above central difference formula for the first order derivative of \( f \) at \( x \) and then applying again central difference formula for \( f(x + \frac{1}{2} h) \) and \( f(x - \frac{1}{2} h) \) we obtain the central difference approximation of the second derivative (one-dimensional laplacian) of \( f \):

\[
\nabla^2 f = \frac{f(x + h) - 2f(x) + f(x - h)}{h^2} \tag{5}
\]

The three-dimensional form of the laplacian in C-IMSSIM for the concentration of chemokines becomes

\[
\nabla^2 c = \frac{1}{dx^2} \sum_{\hat{i}, \hat{j}, \hat{k} \in \{+1,0,-1\}} (c[x + \hat{v}] - c[x]) \tag{6}
\]

where \( \hat{i}, \hat{j}, \hat{k} \) are the unit vectors (i.e. \( \hat{v} \) is the direction of the nearest-neighbor, see Fig.2).

To solve numerically the Eq. 1, an accurate schema would consider a finer grain for the mesh (i.e. using a smaller \( dx \)) with respect to the agent based one (Fig. 1). However, we empirically decided (since we did not find significant differences) to integrate Eq. 1 with the same lattice unit as for the agent based mesh (i.e. \( dx = 1 \)).

Zero-flux boundaries are generally assumed, meaning that cells can not exit from the lymphnode (derivative is zero on the boundary). Optionally, we can set absorbing boundary conditions such that chemokines are absorbed by the boundary.

Equation 6 is valid for an internal point of the lattice. When one of the neighbors lies outside the lattice, the expression of the corresponding summation term changes according to the adopted boundary conditions: zero-boundary: \( c[x + \hat{v}] = 0 \); no-flux boundary: \( c[x + \hat{v}] = c[i] \).

For the internal lattice points, the integration algorithm for Eq. 1 is:

```java
for(x=0; x<dim; x++){
    laplacian=0.0;
    for(v=0; v<6; v++){
        laplacian += c[x+v]-c[i];
    }
    temp[x] = c[x] + D*dt*laplacian
              - k*dt*c[x];
}

for(x=0; x<dim; x++){
    c[x] = temp[x];
}
```

Then, cells move towards increases of chemokine’s gradient (i.e. highest concentration). The corresponding piece of code (for the internal lattice points) follows.

```java
totc=0;
for(v=0; v<6; v++){
    cv[v]=0;
    /* positive part of the gradient */
    if (c[x+v]-c[x]>0) cv[v] = c[x+v]-c[x]
    else cv[v] = 0;
    totc += cv[v];
}

/* if no chemotaxis then move randomly */
if(totc>0){
    for(v=0; v<6; v++){ c[x+v] /= totc; }
    v = RandomWheel(cv);  
} else{
    v = Randint(0,6);
}
```
In words, we first compute the normalized difference of concentration between the current cell position x and the neighboring lattice points x + v, then, the direction v is chosen stochastically by means of a random wheel selection (i.e., on “average” it moves toward the maximum increase of chemokines concentration). If no chemokines are present then the cell follows a random walk.

On top of chemotaxis/diffusion, volume constraints are introduced checking for physical occupancy in the volume corresponding to a lattice point.

Differences in cells mobility are taken into account and typical diffusion times differ from entity to entity (for instance, T cells move faster than B cells [11, 10]). This distinction in cell mobility is important and plays a major role during, for example, an inflammation, as neutrophils are always the first to reach the inflammation site, followed by macrophages and finally by lymphocytes. This is obtained by having, at each time step, a cell to diffuse with a probability proportional to the diffusion coefficient.

This detailed description of the lymphocytes traffic and of chemotaxis phenomena acquires great importance to the light of a more realistic description of the human immune system. For instance ellipsoid-like meshes will be connected (by means of narrow cylinders) to represent lymph nodes connected by lymphatic vessels.

3. HIV infection

AIDS is a form of immunodeficiency caused by the HIV. Its devastating effect in humans is due to the fact that HIV undermines the base of the immune system, since its major target is a class of white blood cells known as CD4+ T which are pivotal in the activation of both humoral and cell-mediated immune response. Because of the central role of CD4+ T cells in immune regulation, their depletion resulting from HIV activity, impairs the ability of the immune system to cope with other pathogens leading to the immunodeficiency status.

Once HIV enters the body, the virus starts to infect CD4+ T cells and replicates inside them. Those new viruses are released into the blood either by budding or when the host-cell dies and keep on infecting other CD4+ T cells. As CD4+ T cells are attacked and destroyed by HIV, the immune system becomes less effective in fighting infections and diseases. In Fig. 3 we report a schematic picture of the time evolution of HIV infection and of the onset of AIDS [6]. The first contact with the virus induces a normal primary immune response (viremia peak), very similar to any other infection, which lasts a couple of months (acute phase). This acute phase is followed by a long period of “apparent” low-activity of the virus (clinical latency) which lasts from 2 to 8 years. When the CD4+ T cell count drops to about 20% of the normal value, there is the onset of a highly immunodeficient status (AIDS). At this time the immune system becomes unable to defend its host from opportunistic diseases and the patient dies within 2-3 years.

To date there is neither a vaccine nor a prophylaxis which can guarantee the immunity from HIV and the search for a vaccine has become part of the struggle against the disease. One of the reasons of the difficulty in fighting the HIV is that the destruction of the CD4+ T cell compartment is not completely understood. The depletion is likely caused by a combination of two main processes: destruction of mature CD4+ T cells by direct and indirect action of the virus, and the impairment of new T cells production. Today only treatments aimed at delaying the diffusion of the virus exist. Highly Active Anti-Retroviral Therapy (HAART) is a cocktail of three or more powerful anti-HIV drugs, commonly Reverse Transcriptase Inhibitors (RTIs) and Protease Inhibitors (PIs). Although most studies agree that HAART is not able to eradicate the virus, it is very successful to allow the stabilization of patient’s symptoms and viremia.

In a recent work it was shown how C-IMMSIM had been enhanced with respect to the CS-model to describe HIV-infection [3]. The main result of that paper is that C-IMMSIM is able to reproduce the peculiar “three-phase” dynamics of AIDS development (described in Fig. 3) and that the simulated progression of the disease in untreated patients is in good qualitative and quantitative agreement with clinical findings [8, 12].

Hereafter we describe the most recent results obtained with C-IMMSIM related to the simulation of the HAART
and in particular we report data about the efficacy of the treatments in controlling the growth of the virus. The simulated life cycle of the virus is represented by the following stages:

1. the virus infects CD4+ T cells, macrophages (MA), dendritic cells (DC);
2. reverse transcriptase copies the viral single stranded RNA genome into a double-stranded viral DNA. The viral DNA is then integrated into the host chromosomal DNA;
3. the virus remains at rest until an event activates the transcription;
4. the replicating virus buds from the cell membrane. Fully assembled virions are then able to infect other cells to start the life cycle again.

The HAART composed by transcriptase and protease inhibitors influences the life cycle of the virus as follows:

- RTIs block reverse transcriptase’s enzymatic function and avoid completion of synthesis of the double-stranded viral DNA thus preventing HIV from multiplying. In the life cycle of HIV this prevents the virus in stage 1 from reaching stage 2 above described;
- PIs prevent viral replication by inhibiting the activity of HIV-1 protease, an enzyme used by the virus to cleave nascent proteins for final assembly of new virions. Thus PIs act in stage 4 by preventing virus assembly.

In our model, two additional bit strings have been included in order to represent the efficacy of the transcriptase and protease inhibition due to the HAART.

It is important to understand that the efficacy of the therapy changes continuously during a simulation as a result of virus mutations. For such reason it is interesting to describe the resistance to the therapy of different viral strains and also possible failures of the treatment.

In Fig. 4 the viral load and CD4+ T cells count are shown. In the upper panel of Fig. 4 we report a sketch of the theoretical behavior adapted from [8]. In the lower panel we report the results of two thousands simulations showing the effects of an anti-retroviral treatment. Each simulation corresponds to a volume of 100µl of lymph node representative of a single patient. Results are in line not only qualitatively but also quantitatively with clinical observations [8].

In the inset of Fig. 4 we focus on the first six months from the primary infection that correspond to the clinical acute phase. The peak of the plasma viral load is achieved within 8 weeks from the infection and correlates directly with post-set-point plateau levels of virus that is \( \log_{10}(\text{vRNA}) \approx 4.75 \) copies/ml and is reached in 12 weeks. All these data are in very good agreement with clinical observations [8]. CD4+ T cells, as widely discussed above, are the main target of the HIV-1 virus. The immune system responds in the first 10 days from infection and the long-lasting depletion of the CD4+ T cells starts after 10 weeks from infection (see inset).

The indications for the beginning of the therapy are based on clinical assessments of CD4+ T cell count and viral load: a lower CD4+ T cell count in conjunction with a higher viral load indicates a higher risk of opportunistic infections that makes more urgent to start the treatment.

In order to tune the model parameters we performed simulations in which the HAART starts during the advanced stage of the clinical latency when the viral load is at the set-point plateau level and the CD4+ T cell count reaches a level consistent with current guidelines (e.g. 250-300 cells/µl) [9].

**Figure 4. Viral load (black line) and CD4+ T cells depletion (grey line) in HIV-infected individuals. The upper panel is adapted from ref. [8]. The average over two thousands simulations is shown in the lower panel.**
As the therapy starts, CD4+ T cell count progressively increases from pathologic low values to physiologic normal values. The model has been tuned to reach an immune reconstitution of about 150 cells/μl after one year of therapy [5]. This value is only indicative since although the virological effect of HAART, that is the suppression of viral replication, is highly reproducible, the degree of immune restoration is variable among different patients and is difficult to predict [5].

During the anti-retroviral treatment the viremia is suppressed below the level of detection ($\log_{10}(50) \sim 1.7$ HIV-1 RNA copies/ml). The decline of initial viremia occurs in two phases: the first phase (first two days after the beginning of HAART therapy) where there is an exponential fall of the viremia of about two orders of magnitude [13]. This rapid ablation of initial viremia reflects the decay of productively infected cells due to the action of RTIs that completely block infection of new cells. The second phase, that starts two days after the beginning of the therapy, is slower because it is due to the action of the PI's that act on cells what were infected before the beginning of treatment. Finally, this decline levels off because of sub-optimal drug effectiveness, reservoirs of virus-producing cells that are not affected by the drug, or the emergence of resistant virus strains [9]. Some of these viral mutations allow the HIV to resist the effects of certain anti-HIV drugs (called “drug resistance”). Anti-retroviral treatment fails to completely eliminate HIV from infected individuals and cessation of HAART results in a rapid viral rebound in line with clinical observations.

4. Conclusions

Our study may acquire great importance in view of new possible strategies for the optimization of the therapy. In particular there exists a discussion about the optimal time for starting the therapy. For instance the so called early therapy applied within the first three weeks of infection seems to be highly efficient in the immune control of the virus and provides a long-term suppression of viral load below the level of detection that seems to withstand also after HAART is terminated.

In a recent work we have used this model in combination to genetic algorithms to find an optimized therapeutic schedule that maximizes drug effectiveness while reducing the dosage [2].

Availability

For demonstration purposes it is possible to start simulations from a browser by using the web-service version available from www.immungrid.org/simulator.

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