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NWA A DISCRETE STOCHASTIC SIMULATION TECHNIQUE: A REVIEW

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ABSTRACT: We give the description for the Nondeterministic Waiting Time (NWT) algorithm, a biochemical modeling approach based on the Membrane Systems paradigm of computation. This simulation technique provides a unique perspective on the biochemical evolution of the cell different from Gillespie's algorithm and different from ODE-based simulations. That is, depending on the reactions and molecular multiplicities of a given model, our simulator is capable of producing results comparable to the alternative techniques - continuous and deterministic or discrete and stochastic. Some results for a few models are given, illustrating the differences between the NWT algorithm, the Gillespie algorithm, and the solutions to systems of ordinary differential equations. We are able to show that in some cases the NWT technique yields results closer to Gillespie, while for other models the results of the NWT simulator are much closer to the results of the ODE simulator.

Keywords: Discrete simulation, Gillespie's algorithm, cellular pathways, deterministic simulation, nondeterministic waiting time simulation with memory

INTRODUCTION AND MOTIVATION OF STUDY

For the design of the NWT algorithm (Jack *et al.*, 2007), we have chosen a biologically inspired underlying framework: Membrane Systems (or P Systems). The essential design goal was to create a new simulation technique capable of exhibiting qualities comparable to the stochastic methods - e.g., the Gillespie algorithm (Gillespie, 1976), (Gillespie, 1977) - but also systems of ordinary differential equations, depending on the particular configurations of the systems being modeled. Moreover, the NWT algorithm is designed to be less computationally intensive than the Gillespie algorithm; however, NWT maintains a level of nondeterminism that allows divergent solutions compared with systems of ordinary differential equations.

The work of Besozzi is in the area of applying variations of Gillespie's algorithm (with τ-leaping) to bacterial chemotaxis or mechanosensitive channels. The main idea of the current paper is to consider a trade-off between speed and sensitivity of the simulation method to be able to reach whole cell simulations. One of the models/examples that we consider in this paper was previously considered in the area of Membrane Systems (but in another setting/framework) in (Fontana & Manca, 2008) considering metabolic networks, which yielded surprising results. We applied our modelling technique to study the latency of the HIV virus.

The HIV virus has several remarkable features: 1. it **infects mostly the immune cells**, 2. shows a **high genetic variation** throughout the infection in a single individual due to the high error rate in the reverse transcription and, finally, 3. it is **inducing cell suicide** in the "healthy" immune cells. Furthermore, because of the way the immune system works we are also faced with the problem of latently HIV infected T cells (reason 1.). It is widely believed that the latently infected T cells represent the last barrier against the HIV cure. The number of latently infected cells in a cell population is rather small, around 3%, which makes the experimental study of these cells difficult (the current technology in biomolecular experimental area requires large numbers of copies of the molecule/cell studied). Because all these reasons the current paper represents an initial modeling effort for the latently infected T cells. We are focussing on the apoptotic modeling (reason 3.) since it is the avenue through which the virus is effectively removing the immune system of the host. We will base our model on the previous modeling work of (Hua *et al.*, 2005), using the simulation technique reported in (Jack *et al.*, 2008). Furthermore, due to the high genetic variability (reason 2.) of the viral proteins we will combine several similar processes together in a single reaction (and also making the modeling work easier). The kinetic constants for the new reactions modeling the interaction of the viral proteins with the host cell will be gathered from the literature, or obtained by model fitting the constants for reported experiments on the non-latent cells. Finally, having the model and kinetic constants settled for the non-latent model, we proceed to simulating the latent cells (immediately after they are reactivated) by changing the initial conditions of the simulation, as well as changing the appropriate rules in the model. We will then proceed to study the predicted behavior of the latently infected cells. In the following we will give a more detailed description of all these steps mentioned so far.

As far as we know, this paper reports the first attempt at modeling the Fas-mediated apoptotic signaling pathway in reactivated latently infected CD4+ T cells. The human immunodeficiency virus type 1 (HIV-1), which is considered to be responsible

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for the pathogenesis of AIDS, has been called a global epidemic. Since its discovery over two decades ago, the HIV-1 has been the target of aggressive research. And yet, a cure for AIDS - total eradication of the viral infection - is still out of reach. According to the World Health Organization, there were 33.2 million people living with HIV in 2007, 2.5 million newly infected individuals, and 2.1 million AIDS deaths: *World Health Orgnization*.

The pathogenesis of AIDS is attributed to the depletion of the host's CD4+ T cells, the loss of which results in a dysfunctional immune system. Finkel *et al.*, In (Finkel *et al.*, 1995) concluded that HIV-1 infection causes death predominantly in bystander T cells. These healthy, uninfected T cells are marked for destruction by the neighboring HIV-1-infected T cells. These cells appear to primarily undergo apoptosis, or programmed cell death. It seems that proteins encoded in the HIV-1 genome exhibit anti- and pro-apoptotic behavior on infected and bystander cells, enhancing or inhibiting a cell's ability to undergo apoptosis. There are numerous drugs available for limiting the impact of HIV-1 on the immune system; the most successful approach, highly active anti-retroviral therapy (HAART), is a combination of several types of drugs, targeting different mechanisms of HIV-1 infection and proliferation.

Although HAART has proven to be effective in the reduction or elimination of viremia (Perelson *et al.*, 1997), it is ineffective in the complete eradication of the viral infection. Latent reservoirs of HIV-1 have been detected HIV-1 infected patients (Chun *et al.*, 1995), (Chun *et al.*, 1997). Latently infected cells are relatively rare - about 1 in 10^6 resting T cells (Chun *et al.*, 1997). However, they are considered to be the largest obstacle in combating HIV-1 infection (Finzi *et al.*, 1999, Siliciano *et al.*, 2003) (Strain *et al.*, 2003). Understanding the mechanisms behind HIV-1 latency is a focal point for current AIDS-related research (for a recent review on latency see (Han *et al.*, 2007).

There are two types of latency described in the literature. The first, preintegration latency, refers to resting T cells containing unintegrated HIV-1 DNA. Since the unintegrated HIV-1 DNA is labile and reverse transcription of HIV-1 RNA is slow (on the order of days (Zack *et al.*, 1990) (Zhou *et al.*, 2005) (Zack *et al.*, 1992, Pierson *et al.*, 2002), it is believed that patients with reduced viremia after several months of HAART therapy do not have resting T cells with unintegrated HIV-1 DNA (Blankson *et al.*, 2000). However, resting T cells with stably integrated HIV-1 DNA can provide a reservoir for viral reproduction for years (Finzi *et al.*, 1999). These reservoirs are the result of activated HIV-1-infected T cells that have returned to a quiescent state. Due to their long lifespan, we have chosen to model the apoptotic events that follow the reactivation of a post-integration latently infected CD4+ T cell. N.B., for the remainder of the paper, when use the term latent, we are referring to the post-integration latency.

We have previously reported a model of the Fas-mediated apoptotic signaling cascade, which mimics the behavior of the Jurkat T cell line (Jack *et al.*, 2008). In (Jack *et al.*, 2008), we provided an exhaustive study on the feasibility of our Nondeterministic Waiting Time (NWT) algorithm, comparing our results to an established ordinary differential equations (ODEs) technique (Hua *et al.*, 2005). We have extended the Fas model, incorporating the effects HIV-1 proteins have on the pathway.

In Section 2, we provide a brief outline of our simulation technique as well as a discussion on the background information on the Fas pathway and HIV-1 proteins used to build our model. Section 4 contains the results of our simulations. Finally, Section 5 is a discussion of issues revolving around modeling HIV-1 protein activity and future research plans for our group.

MATERIALS AND METHODS

The NWT Algorithm

We refer the interested reader to (Jack *et al.*, 2008), where we provided a detailed description of the NWT algorithm. We will highlight the key aspects of our simulation technique.

The NWT algorithm is a Membrane Systems implementation, where the alphabet of the system is defined as proteins, and the rules are the reactions involving the proteins. The algorithm is mesoscopic, since individual molecules are employed instead of molecular concentrations. This allows us to discretely interpret the evolution of the intracellular molecular dynamics. We have argued in (Jack *et al.*, 2008) that our discrete, nondeterministic algorithm may outperform other nondiscrete methods - for example, ODE simulations.

All of the reactions within the system obey the Law of Mass Action - i.e., the amount of time required for any given reaction to occur is directly proportional to the number of reactant molecules present in the system. The Law of Mass Action is used to calculate a waiting time (WT) for each reaction, indicating the next occurence of the reaction. These values are based on kinetic constants and are deterministic - the nondeterminism in our algorithm stems from reaction competition over low molecular multiplicity. Our NWT algorithm is different than the Gillespie algorithm (Gillespie 1976, 1977), where stochastic values are generated to govern the reaction rates. We have used the NWT algorithm to explore the effects of HIV-1 proteins on the Fas-mediated signaling cascade.

The Membrane System is a mathematical description of the entire biochemical model, defining the reactions, cellular compartments, molecular species and multiplicities, etc. With this formalism in place, we will now provide the steps for the NWT algorithm.

I. *Build Membrane System*: Import information for Membrane System - alphabet, membrane hierarchy, etc. Convert protein concentrations to molecular multiplicities. Convert kinetic rates to discrete kinetic constants. For each reaction R_i, where

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$1 \le i \le m$, we calculate the initial Waiting Time, WT_{R_i}. Choose the desired amount of time for the simulation, τ_{fin}. Set current simulation time to zero ($\tau=0$).

II. *Build Heap:* Using the reaction Waiting Times, we build a min-heap of all reactions in the system.

III. *Select Rule:* Choose the reaction with the lowest Waiting Time -- the top of the min-heap. Assign now the value for the next step in the simulation: $WT_{applied}$ as the time stored in the root of the heap. Upon selecting the top node, recursively check to see if there are any children nodes sharing the minimum Waiting Time. If such a tie for minimum Waiting Time exists, then proceed to Step IV. If no tie exists, then continue to Step V.

IV. *Handle Tie:* Check the multiplicities of the reactant species for all tied reactions. If there are enough reactants to satisfy all of the reactions with the minimum Waiting Time, implement all tied reactions. If there are not enough reactants to accommodate all the reactions, use the nondeterministic logic to apply as many rules as possible.

V. *Apply Rule:* Update the multiplicities of the reactant(s) and product(s) for the reaction(s) from Step III. Aggregate the simulation time ($\tau = \tau + WT_{applied}$).

VI. *Update Rules:* Recalculate the Waiting Time for all reactions whose reactant(s) include the product(s) or reactant(s) of the applied reaction(s). That is, we need to see how the multiplicity changes from the applied reaction(s) have affected the Waiting Times for each rule dependent on those proteins with a new multiplicity. For each such reaction update the old Waiting Time with the newly computed Waiting Time (according to the new multiplicities in the system and the memory enhancement). Of course, the new time could be infinity, indicating insufficient reactants for that reaction to occur.

VII. *Memory Enhancement:* If the recalculation of a reaction's Waiting Time results in a value of infinity, then we must store the amount of time waited as a percentage (Mem_{perc}). If the recalculation of a reaction's Waiting Time results in a real value and the previous value was infinite, then the Waiting Time will need to be adjusted according to the stored memory percentage.

VIII. *Heap Maintenance*: Adjust the min-heap, bubbling reaction nodes up or down in order to satisfy the min-heap property, once reaction Waiting Times have been recalculated according to the multiplicity changes. N.B., to accommodate the multiple changes in Waiting Times, we employ nonstandard heap maintenance methods.

IX. *Termination:* If $\tau=\tau_{fin}$, then terminate the simulation. Output the multiplicity information for entire simulation. Otherwise, go back to Step III.

Our simulation technique is modular; we initialize the NWT with models encoded in the Systems Biology Markup Language (SBML). SBML is one of the most popular methods to encode biochemical models. It is developed through a broad international collaborative effort with many cooperating institutions (Hucka *et al.*, 2003). To set up the SBML files, we use the CellDesigner software (Funahashi *et al.*, 2003, Funahashi *et al.*, 2008), which is maintained through Keio University.

With the SBML code, and the kinetic constants/WT calculations described earlier, we have the information for the initialization of the system. This sufficiently describes Step I of the NWT algorithm. In ANSI C, we can store all of the information describing the Membrane System in two arrays of structs: an alphabet array and a rules array. For Step II, we build a min-heap in the standard bottom-up way. For any two reactions, R_1 and R_2, in the heap, if R_2 is a child of R_1, we have $WT_{R_1} \le WT_{R_2}$.

Step III of the NWT algorithm, selecting a rule, requires O(1) time to complete, since the WTs were sorted in Step II. However, we must check to see if any other rules have the same WT. If two or more rules are attempting to execute at the same instant of time, we must ensure there are enough reactants to execute all competing rules. If insufficient numbers of molecules exist for all the potentially executable rules, then we must nondeterministically apply reactions until all available reactants have been exhausted. We create an array pointing to all rules slotted to occur at the next moment in time. If there is only one element in the ties array - no competition for resources - we can move on to Step V, skipping Step IV, and execute one rule. Otherwise, we proceed with Step IV.

To nondeterministically apply rules, we randomly generate numbers between 0 and the end of the ties array. Using this randomly chosen index, we check if sufficient reactants exist to implement the reaction. If there are sufficient reactants, we apply the reaction - i.e., we increase the multiplicity of the product(s) by one and decrease the multiplicity of the reactant(s) by one. If there are insufficient reactants, we skip the reaction, and no multiplicity changes occur for the reaction. In either case, the reaction is removed from the ties array, and the process continues until the ties array is empty. This completes the discussion for Steps IV and V of the algorithm. Remember, in the case of only one reaction, we skip Step IV and apply just the one reaction in Step V. Either way, we are ready to move on to Step VI: Update Rules.

For each reaction applied in Step V, we must recalculate the WT of the applied reaction and the WT of every reaction affected by the multiplicity changes. We must discuss Step VI within the context of the heap maintenance. Hence, we will continue the discussion of Step VI later, which will continue with the discussion of Step VIII, Heap Maintenance. As you will see in the next two Sections, Steps VI, VII, and VIII are intertwined.

The model: Fas-mediated apoptosis

We have explored the literature pertaining to the effects of HIV-1 proteins on apoptosis: see (Ross 2001, Selliah & Finkel 2001, Alimonti *et al.*, 2003) for reviews on HIV-1-related CD4+ T cell death. There are several distinct death receptors, which, upon

activation of the cell, can lead to cellular apoptosis through a tightly regulated molecular signaling cascade (Ashkenazi & Dixit 1998). In this paper, our concern is the Fas pathway. As reported in (Rieux-Laucat *et al.*, 2003) and (Igney & Krammer 2002), understanding the complex signaling cascade of Fas-mediated apoptosis can be beneficial in developing remedies for cancer and autoimmune disorders. Several of the HIV-1 proteins have been implicated in the Fas-mediated signaling cascade.

In (Scaffidi *et al.*, 1998), the authors describe two signaling pathways for Fas-mediated apoptosis: type I and type II. Both pathways begin with the Fas ligand binding to the Fas receptor (also called CD95) on the cell membrane. This results in a conformational change at the receptor, producing a complex, Fasc. The cytoplasmic domain of this complex recruits Fas-associated death domain (FADD). A maximum of three FADD molecules can be recruited to each Fasc molecule. Once FADD is bound to Fasc, Caspase 8 and FLIP are recruited competitively. Although three molecules of Caspase 8 can be recruited to each Fasc-FADD binding, only two are required to create the dimer, Caspase $8_2^{P_{41}}$, which is released into the cytoplasm. The cytoplasmic Caspase $8_2^{P_{41}}$ is then phosphorylated into active form (Caspase 8*). The binding of FLIP to Fasc is an inhibitor of apoptosis, because it reduces the ability of Caspase 8 to become activated - i.e., FLIP attempts to occupy the necessary binding sites.

Unless sufficiently inhibited, the Fas signaling cascade continues in the type I or type II pathway. For sufficiently large Caspase 8 initial concentration, Caspase 3 is directly phosphorylated by the Caspase 8*. This is the type I pathway. If the number of Caspase 8 molecules is insufficient to induce Caspase 3 activation directly, the type II pathway initiates. Caspase 8* truncates the Bid protein, tBid. The tBid binds to Bax molecules with a one to two ratio. The result of the bound Bax and tBid is the release of Cytochrome c from the mitochondria. Once it is translocated to the cytoplasm, Cytochrome c binds to Apaf and ATP, forming a complex that can recruit and activate Caspase 9 (Caspase 9*). The activated Caspase 9* proceeds to activate Caspase 3. We consider the activation of Caspase 3 to be the end of the signaling cascade. For other details on Caspases 8 and 3 we refer the interested reader to (Bratosin *et al.*, 2009).

In (Jack *et al.*, 2008), we modeled both the type I and type II Fas-induced apoptotic signaling pathways. In the next section, we provide will discuss HIV-1 infection and its effects on the Fas signaling cascade.

HIV-1 Infection

The mechanisms behind HIV-1 infection of CD4+ T cells are well understood. A spike on the virus, gp120, binds to the CD4 receptor of primary T cells, and in conjunction with subsequent binding to a coreceptor (CCR5 or CXCR4), a path is opened for the virus to inject its contents into the cell (Chan & Kim, 1998; Wyatt & Sodroski, 1998). Reverse transcriptase creates cDNA from the HIV-1 RNA and the genome of the virus is implanted into the cells own DNA for future production, as long as the cell remains activated. During this time, the immune system fails to detect and destroy the infected cell.

There is still some debate about the effects of HIV-1 proteins on cellular signaling networks; however, we have pooled the collective knowledge of the biological community in order to categorize and model the described functions of various HIV proteins. For an illustration of the Fas pathway and the involvement of the HIV proteins, we refer the reader to Fig. 1.

Besides the viral RNA, the infecting virions contain proteins which interact with various signaling cascades of the host cell -- for example, the Fas pathway. By doing so, the HIV-1 proteins prepare the cell for viral reproduction while avoiding annihilation by the immune system. It is not until the immune system activates the T cell that viral reproduction occurs. We will describe the molecular multiplicities within the virion when we discuss the rules of each protein later in the section.

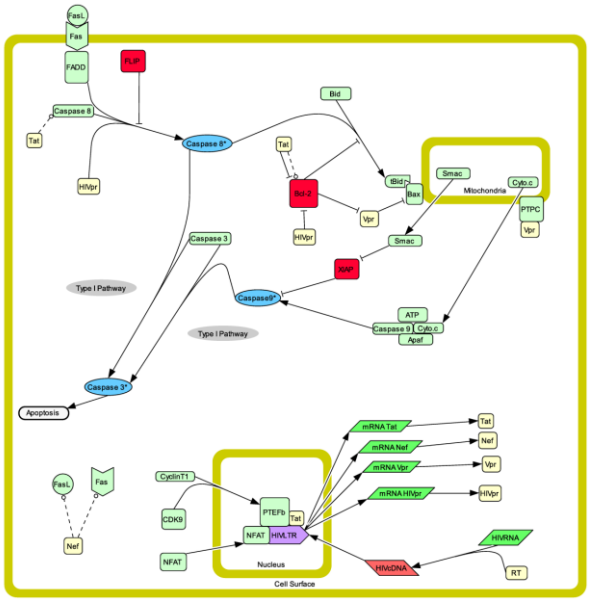

Figure 1. We consider several mechanisms for HIV-1 protein effects on Fas signaling. The cleavage of Procaspase 3 is the end of the signaling cascade - which irrevocable leads to cell death. The type I pathway involves direct activation of Procaspase 3 by Caspase 8. The type II pathway requires signal amplification through the release of cytochrome c from the mitochondria resulting in Caspase 9 activating Procaspase 3. The HIV-1 Tat protein upregulates Procaspase 8 and Bcl-2, but it can also down-regulate Bcl-2. Vpr upregulates Bcl-2 and downregulates Bax. HIV protease can cleave Bcl-2 into an inactive form and it can also cleave Procaspase 8 into active Caspase 8.

Effects on Fas pathway

Aside from its role in transcriptional elongation, the Tat protein is responsible for both pro- and anti-apoptotic behavior. In (Bartz & Emerman, 1999), the authors were able to demonstrate that increased Tat expression causes upregulation of Caspase 8. Also, Tat has been associated with the downregulation of Bcl-2 (Selliah & Finkel, 2001). Given the pro- and anti-apoptotic duties of Caspase 8 and Bcl-2, respectively, it appears that a cell with high levels of Tat has increased susceptibility to apoptosis. Conversely, (Finkel *et al.*, 1995) claims that Tat upregulates Bcl-2, resulting in decreased apoptotic rates of cells. Tat has also been implicated in the upregulation of Fas ligand on the cell surface (Bartz & Emerman, 1999) (Yang *et al.*, 2002), which may effect the cell through autocrine signaling.

We have implemented two pro-apoptotic rules for the Nef protein. (Zauli *et al.*, 1999) discovered that Nef may play a role in regulating cell death by upregulating Fas receptor and Fas ligand on the cell surface. Upregulating the receptor sites of Fas on the cell surface prepares the cell for ligand binding, and can initiate the Fas-induced apoptotic signaling cascade. The upregulation of Fas ligand may protect the infected cell from cytotoxic T cells, or it could be part of autocrinic signaling. There are four rules for upregulation and translocation of Fas and Fas ligand.

RESULTS AND DISCUSSION

We added all of the rules mentioned before to the Fas model described in (Hua *et al.*, 2005; Jack *et al.*, 2008). To see the complete list of rules, see Appendix A. For the remainder of the paper, we refer to a „*nonlatent*" cell as an activated T cell that has just been infected with HIV-1. The HIV-1 RNA and other virion proteins are in the cytoplasm. The HIV-1 RNA must be incorporated into the host's genome before the HIV-1 proteins can be produced. We refer to „*latent*"' cells as newly activated T cells with no HIV-1 proteins present, but the HIV-1 genome has already been implanted in the host's DNA.

We used the *nonlatent* cell for the model fitting, since the majority of information about HIV-1 proteins pertains to these types of cells. For instance, in Fig. 2 (A), we see that high levels of Tat upregulates Caspase 8 by 3-fold. This agrees with the observed results in (Bartz & Emerman, 1999). Also, in Fig. 2(B), our simulations indicate that high levels of Vpr lead to the upregulation of Bcl-2 and downregulation of Bax by 20% and 30%, resp. These results concur with the results shown in (Conti *et al.*, 1998).

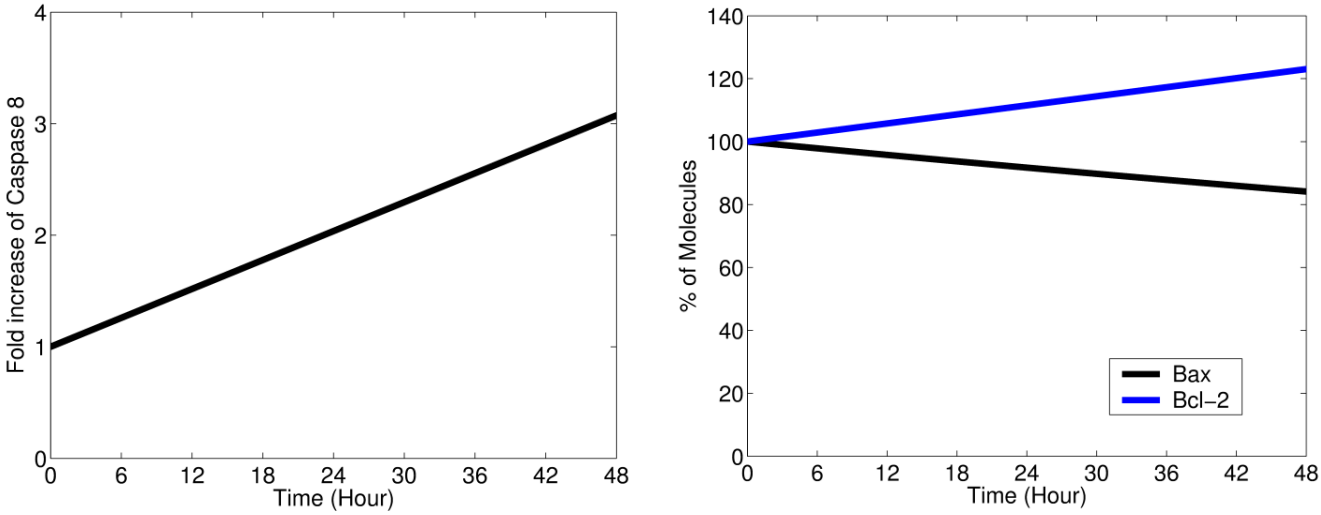

Figure 2. Left: High levels of Tat protein upregulates Caspase 8 levels by three fold. Right: High levels of Vpr upregulates Bcl-2 and downregulates Bax by 20% and 30%, resp.

We mentioned earlier that the end of our signaling cascade is the activation of Caspase 3. In Fig. 3 we see that both the *nonlatent* and *latent model* cells start show the onset of apoptosis - total activation of Caspase 3 - after approximately two days. Our simulations do indicate that reactivated latently infected CD4+ T cells die activate all of the Caspase 3 earlier than the *nonlatent* model. The reason for this might be that the latent cells lack the initial Vpr molecules, since the anti-apoptotic effects of the protein would not be available. Also in Fig. 3 we show the truncation of Bid, a necessary step in the induction of the type II pathway. Active Caspase 8 is responsible for the truncation of Bid, so we can see the effects of Caspase 8 activation.

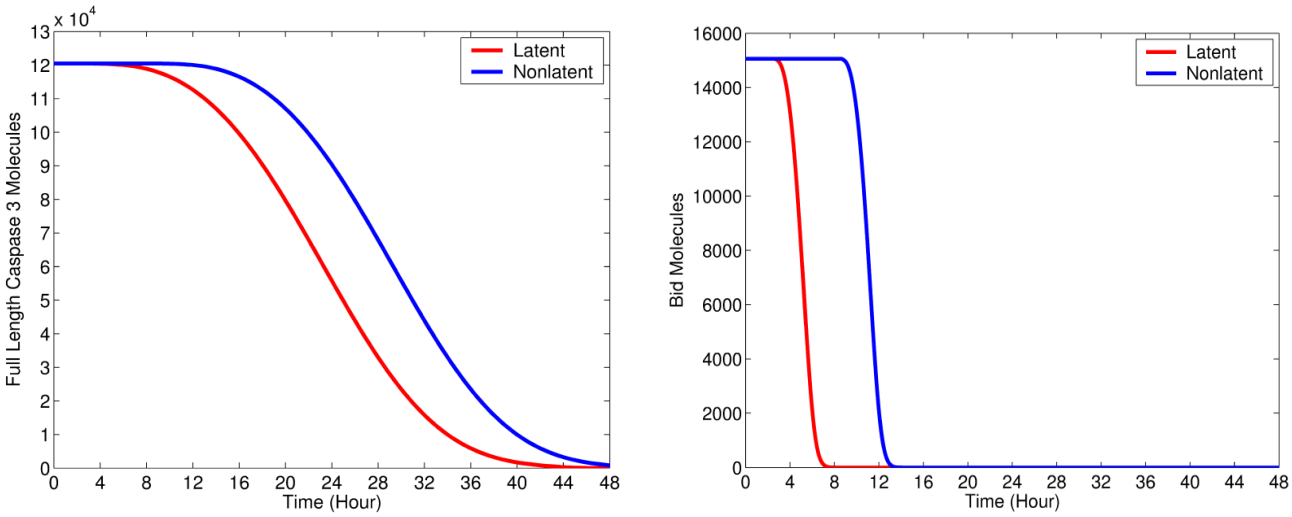

Figure 3. Left: Total reduction of full length Caspase 3 is seen after ~42 hours in the *latent* model, whereas the *nonlatent* model takes ~49 hours. Right: The truncations of Bid molecules are seen, resulting in a reduction in the total number of tBid molecules. This signals induction of the type II pathway.

CONCLUSIONS

Based on the biological evidence in the literature, we were able to construct a simulation of the effects of HIV-1 proteins on the Fas-mediated apoptosis pathway. This is the first computational model of Fas-induced apoptosis in reactivated latently infected CD4+ T cells. With this model, we have attempted to understand CD4+ T cell latency. Interestingly, we have found cooperation between the type I and type II pathways. We have not been able to verify an explanation for this in the current literature.

We are interested in extending this model in several ways. For instance, it would be interesting to model the effects of HIV-1 proteins on bystander cell apoptosis. As mentioned in the introduction, HIV-1 appears to primarily kill uninfected bystander T cells (Finkel *et al.*, 1995). Various mechanisms have been reported for the destruction of the bystander cells. Along with Fas-induced apoptosis, other possible mechanisms for bystander cell death are reviewed in (Ross, 2001; Selliah & Finkel, 2001; Alimonti *et al.*, 2003). Upon being exocytosed by an infected cell, several of the proteins encoded in HIV-1 can exhibit destructive qualities when interacting with neighboring bystander cells - either on the surface or through endocytosis.

There are a few HIV-1 proteins we have ignored in this model, because they affect T cells in ways out of the scope of our current Fas model. For example, soluble and membrane-bound Env can bind to the CD4 receptor of bystander cells. Along with subsequent binding to a co-receptor, it can induce apoptosis. In (Cicala *et al.*, 2000) and (Biard-Piechaczyk *et al.*, 2000), it has been shown that ligation of the CD4 receptor by Env, is sufficient to increase apoptosis in bystander cells. The reasons for increased apoptosis following Env-CD4 binding can be attributed to Bcl-2 down-regulation (Hashimoto *et al.*, 1997), increased Caspase 8 activation (Algeciras-Schimnich *et al.*, 2002), and upregulation of Fas (Oyaizu *et al.*, 1994), FasL and Bax (Selliah & Finkel, 2001).

Extracellular Tat can be endocytosed by a bystander cell, resulting in pro-apoptotic behavior. The addition of Tat to a culture of uninfected cells has been shown to increase apoptosis (McCloskey *et al.*, 1997). Endocytosed Tat can up-regulate levels of Caspase 8 (Bartz & Emerman, 1999) and increase expression of the Fas ligand (Selliah & Finkel, 2001), similar to its effects in infected cells. Extracellular Vpr can disrupt the mitochondrial membrane, leading to increased translocation of cytochrome c* (Selliah & Finkel, 2001).

Finally, we would like to note that the experimental information on the latent HIV-1-infected T cells is scarce, due to the fact that these cells are found in such small numbers *in vivo*. Therefore, our model relies heavily on applying the knowledge of activated HIV-1-infected CD4+ T cells. We look forward to new experimental results about these enigmatic cells, which we can use to refine our model.

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