Evolving Boolean Networks to Find Intervention Points in Dengue Pathogenesis

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ABSTRACT
We use probabilistic boolean networks to simulate the pathogenesis of Dengue Hemorraghic Fever (DHF). Based on Chaturvedi's work, the strength of cytokine influences are modeled stochastically as inducement probabilities. Two basins of attractors are observed with synchronous updating; the Null Infection cycle attractor shows an expected cross-regulation of Th1 and Th2 cytokines corresponding to the homeostasis of an uninfected person, while the DHF Infection attractor shows the onset of DHF. With asynchronous updating, our model remains valid with clinical comparisons against qualitative changes in signal durations. In order to find intervention points that could prevent DHF, we design a genetic algorithm to shift the DHF attractor to the DF attractor basin by using the DF final state as the fitness measure. Our simulation results identify TGF-β, IL-8 and IL-13 as the intervention points which are consistent with known clinical results to prevent DHF from occurring.

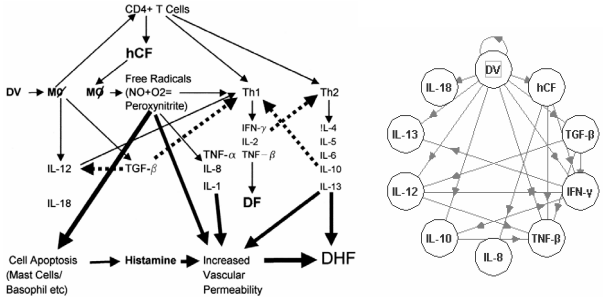
Categories and Subject Descriptors
I.6.5 [**Model Development**]: Modeling Methodologies; J.3 [Life and Medical Sciences]: Biology and Genetics.

General Terms
Algorithms, Measurements, Design, Experimentation.

Keywords: Boolean Network, Dengue Hemorrhagic Fever, Evolutionary Computation, Intervention.

1. INTRODUCTION
The dengue virus is transmitted through the bite of the aedes aegypti/albopictus mosquito, which is also a natural host for the dengue virus, apart from humans. Infection with the dengue virus may lead to Dengue Fever (DF) or the potentially fatal Dengue Hemorrhagic Fever (DHF). Patients with DHF have increased vascular permeability and abnormal hemostasis. This can cause the individual to lose blood volume, result in hypotension, go into shock and die. Researchers [1] have hypothesized that the synergistic effect of the cytokine cascade which shifts a Th1 to a Th2 type immune response causes the said inflammatory characteristics of DHF. Understanding how and when the dynamics of this cytokine cascade will progress to DHF is vital if we are to find a way to defeat the pathogen.

2. DENGUE HEMORRHAGIC FEVER
The main feature of DHF is the early generation of a unique cytokine called human cytotoxic factor (hCF) that initiates a series of events leading to a shift from Th1-type response to Th2-type response. The dengue virus induces the activated T-helper (Th) cells to produce hCF that in turn induces macrophages (M∅) to produce free radicals such as nitrate, reactive oxygen and peroxynitrite. These free radicals will kill target cells by apoptosis and at the same time upregulate the production of pro-inflammatory cytokines IL-1β, TNF-α, IL-8 and hydrogen peroxide in macrophages. Changes in IL-12 and TGF-β will push a Th-1 dominant response to a Th-2 biased response, resulting in exacerbation of the dengue disease. The increased vascular permeability results due to the combined effect of histamine, free radicals and pro-inflammatory cytokines. Figure 2.1(a) illustrates these interactions. The thin lines describe positive induction, dashed lines as inhibition and thick lines as damaging effect. We will subsequently refer to Figure 2.1(a) as the **DHF Cytokine Cascade**.



(a) **(b)**

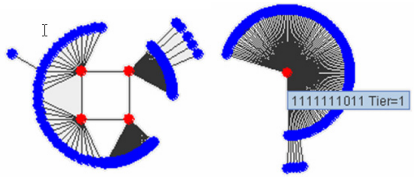
Figure 2.1 DHF Cytokine Cascade (adopted from [1]) and the DHF Infection Model

3. BOOLEAN NETWORK MODEL
We define a probabilistic boolean network G [3][2], where the set of nodes N(G) represents individual cytokines that are known to be significant in the DHF Cytokine Cascade. The edge set E(G) represents directed chemical influences that cytokines have on each other via some intermediate cell or cells (as some cytokines may be produced by more than one type of cell), which we omit from N(G) so as to obtain a pure cytokine-based communication network. Each edge $x \rightarrow y$ in G represents a causal relationship between cytokine x and y, and a boolean function for y will compute its expected likelihood of being assigned a state of 1 or 0 (an ON or OFF state) given all the incident nodes (including reflexive effects). Figure 2.1(b) gives the resulting **DHF Infection Model** constructed based on the DHF Cytokine Cascade. The null version of the DHF Infection Model is obtained through the self-loop at the node DV. This reflexive influence (the only incoming edge) can be either 0 or 1, allowing us to selectively simulate either the **DHF Null Model** or the DHF Infection Model.

4. SIMULATION
We simulate our completed DHF Infection Model (with assigned probabilities) using synchronous updating to verify that stable attractors correspond to clinical predictions. There are two distinct

GECCO'06, July 8–12, 2006, Seattle, Washington, USA.
ACM 1-59593-186-4/06/0007.

attractors observed. The first develops when DV is not introduced and forms our Null Infection Model (see Figure 4.1(a))of a healthy uninfected person while the second forms DHF Infection Model (see Figure 4.1(b)).



(a) (b)

Figure 4.1 Attractor Basins of Null and DHF Infections

We observe that when DV is not introduced, the Th1 and Th2 signals toggle between ON and OFF states in the cycle. This cross-regulation of Th1 and Th2 immune responses is achieved mainly by IL-10 and IFN-γ. Yates, Bergman, Hemmen, Stark and Callard [4] gives a mathematical model to support the hypothesis that this regulation is done in an oscillatory manner, in that secreted levels of Th1 and Th2 cytokines often swing from a high to low value to regulate each other. The attractor when DV is introduced is one that corresponds to the DHF final state. This deterministic model shows a short and stable pathogenesis and the cross-regulation of Th1 and Th2 cytokines is now absent. Without this cross-regulation, the Th2 cells will increase significantly, which Chaturvedi [2] describes as causing increased vascular permeability leading to DHF. Asynchronous updating of nodes, although more biologically realistic, does not identify stable attractors. However, by computing the duration that a node remains in the ON state, we can use relative differences in this duration distribution (for the network) in the Null and DHF Infection Models and compare them with reported strengths of cytokine signals found in patients during DV infection.

Table 4.1 Comparison between BNet results and Clinical Results on the DHF Infection Model

	Null Model (%)	DHF Model (%)	Difference (%)	Difference* (Increase / Decrease)	DHF Clinical Results* [1]
hCF	30.04	70.00	39.96	↑↑	↑↑
TGF-β	30.05	69.94	39.89	↑↑	↑↑
IFN-γ	62.03	62.63	0.6	↑	↑
IL-8	41.99	58.08	16.09	↑↑	↑↑
IL-10	41.45	63.64	22.19	↑↑	↑↑
IL-12	50.97	48.93	-2.04	↓	↓
IL-13	41.28	63.67	22.39	↑↑	↑↑
IL-18	30.09	70.00	39.91	↑↑	↑↑

*↑ Increase, ↑↑ Marked Increase, ↓ Decrease, ↓↓ Marked Decrease

Column 4 and 5 of Table 4.1 shows the quantitative and qualitative differences in cytokine production durations between the Null and the DHF Infection Models when using asynchronous updating. We observe that hCF increases significantly, consistent with Chaturvedi's hypothesis on hCF being the main cause of DF. Also, while Th1 cytokines increased only slightly (<1%), Th2 cytokines, IL-10 and IL-13, increased significantly from 41.45% and 41.28% to 63.64% and 63.67% respectively. These results are consistent with increased Th2 cytokine levels reported to be the direct causes of DHF.

5. FINDING INTERVENTION POINTS

Our purpose is to find the intervention points that allow us to decrease the basin of attraction of DHF by increasing the basin of attraction of DF. We will therefore use the final states of DF (seen in Figure 4.1(b)) as a fitness measure and individuals as mutated instances of the DHF Infection Model.

We conduct GA simulations with 100 generations, at 0.1 mutation rate and 0.01 crossover rate (obtained through experimentation). For each allele, there is a 0.1 chance of mutation and a 0.5 chance of change by 0.1. We use single-point crossover with 0.01 chance of occurrence. Population size was maintained at 30 with Tournament selection and replacement of 10 individuals. The resulting PBN obtained is the **DF Infection Model**. We then identify significant differences between the evolved DF Infection Model and the DHF Infection Model. The significant changes (from comparisons of IP values between PK-Maps for corresponding nodes) represent intervention points in the network and imply changes of cytokine influences. By identifying these points, we can assess the impact of certain cytokines on the shift from a DHF attractor to a DF attractor. By simulating (with asynchronous updating) the evolved DF Infection Model, we verify that the DF final state is commonly occurring. A comparison of the DF Infection Model with the DHF Infection Model show changes that correlates with Chaturvedi's [1] hypothesis that DHF is caused by the increased levels of Th2 cytokines while DF reaction is by increased levels of Th1 cytokines. A comparison of the DF Infection Model with the Null Infection Model in Table 5.1 shows a change in activation levels (hence strength) that correlates with clinical results [1]. Overall, we identify three intervention points; namely, **TGF-β**, **IL-8** and **IL-13**. TGF-β is found to have significant decrease in probability of production (from 0.7 to 0.1) in the presence of DV. This corresponds to clinical results [1] that the maximum levels of TGF-β were detected in patients with DHF grade IV implying a correlation between TGF-β and the severity of DHF.

Table 5.1 Comparing Null and DF Infection Models

	Null Model (%)	DF Model (%)	Difference (%)	Difference* (Increase / Decrease)	DF Clinical Results* [33]
hCF	30.08	99.91	69.83	↑↑	↑
TGF-β	30.15	19.91	-10.24	↓	↓
IFN-γ	61.87	79.69	17.82	↑↑	↑↑
IL-8	42.02	19.99	-22.03	↓↓	↓
IL-10	41.37	34.07	-7.3	↓	↓
IL-12	50.92	85.99	35.07	↑↑	↑↑
IL-13	41.46	12.20	-29.26	↓↓	↓
IL-18	30.08	99.92	69.84	↑↑	↑

*↑ Increase, ↑↑ Marked Increase, ↓ Decrease, ↓↓ Marked Decrease

In the presence of DV, IL-8 also has a significant change (from 0.7 to 0.0) in probability of production. This change supports the hypothesis that increased vascular permeability with leakage of plasma in DHF is associated to the presence of high levels of IL-8. Finally, IL-13 levels also decreases significantly when DV is present (by 0.7 and 0.6 on two influencing node values of IFN-γ). As IFN-γ is a Th2 inhibitor, an increase in the former will reduce levels of IL-13 (see Figure 2.1(a)).

6. REFERENCES

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