Introduction

It is a well-established fact that bacteria play an important role in the ecosystem. Many bacteria are known to have positive effects on human health (Heyman & Ménard, 2002; Guarner & Malagelada, 2003). The most widely known and studied bacteria that have beneficial health effects are from the human gut. They play an important role in digestion, synthesis of vitamins, adsorption of micronutrients as well as in maintaining homeostasis of the immune system (Guarner & Malagelada, 2003). In addition to this, they act as a barrier against a variety of pathogenic/opportunistic bacteria. Several studies have been undertaken to understand the mechanisms by which gut bacteria inhibit the growth of opportunistic bacteria. These studies have reported that non-pathogenic gut bacteria prevent attachment and subsequent entry of pathogenic/opportunistic bacteria in the epithelial cells (Lu & Walker, 2001). Further, gut bacteria compete for nutrient availability and maintain their colonies by consuming all available resources. Thus beneficial bacteria are being used successfully to inhibit and prevent the growth of infectious bacteria. Contrary to the earlier view that bacteria are only harmful, the above-mentioned studies have established the beneficial role of bacteria and have helped to recognize their value in the ecosystem.

At present, most of the viruses are believed to be harmful. Very few studies have reported beneficial roles of viruses (Miedzybrodzki et al., 2005). Most common examples of the inhibition of one virus in the presence of another are of bacteriophages. For example, the bacteriophage λ acts against herpes simplex virus, both DNA viruses (Centifanto, 1965), M13 phage is reported to have antiviral activity against duck hepatitis B virus, both DNA viruses (Iizuka et al., 1994) etc. Such pairs in which one virus inhibits the other can be termed as virus-anti-virus pairs where the word virus is used for that species which infects the humans/animals/plants and causes disease while the infection of anti-virus reduces or neutralises the impact of the virus and is known to be harmless in that species.
Current studies suggest certain common features for virus-anti-virus pairs. These include type of nucleotides, modulation of receptor for entry of virus, type of cells being infected etc. In addition, there might be common features in the mechanism of inhibition of a virus by its antiviral. Such virus-anti-virus pairs will decrease the effective viral load of harmful virus provided its anti-virus does not have undesirable effects in an organism. One such virus-anti-virus pair of Human Immunodeficiency Virus - 1 (HIV-1) and GB virus C (GBV-C) was studied to understand the course of infection of HIV-1 in presence and absence of its antiviral.

A1.1 Human immunodeficiency virus (HIV)

The HIV, a widely studied virus, is a single-stranded, positive-sense RNA virus belonging to the Retroviridae family. It is a member of the Lentivirus genus. The two types HIV-1 and HIV-2 share about 45% sequence homology in their genome. HIV-2 occurs mostly in West Africa, whereas HIV-1 has spread all over the world. The fact sheet released in November 2012 by the Joint United Nations Programme on HIV and AIDS (UNAIDS), reported that there are approximately thirty four million people currently living with HIV infection and out of these around five million people live across South/South-East Asia and East Asia. This makes HIV one of the most wide spread infectious viruses.

HIV targets the cluster of differentiation 4+T (CD4+T) lymphocytes, which are the most abundant white blood cells of the immune system (Nowak & May, 2000). Increase in HIV viral load has been correlated with the depletion of CD4+ T lymphocytes and disease progression. The HIV enters CD4+ T lymphocytes with the help of its envelope (env) protein. The env protein is a trimer of glycoprotein (gp) - gp120 and gp41. The entry process can be divided into three key steps -

i. The virion binds to the host cell through the attachment of CD4 binding domain of gp120 to CD4 protein of CD4+ T cells.

ii. The conformational changes in gp120 protein exposes its chemokine binding domain that enables interaction with C-C chemokine receptor type 5 (CCR5) or C-X-C chemokine receptors type 4 (CXCR4) of CD4+ T cells.

iii. The gp41 subunit of env protein then enables the fusion of viral and host membranes allowing delivery of HIV RNA, reverse transcriptase, integrase, and other viral proteins to the host cell.
Once inside the host cell, HIV goes through following steps to reproduce and create new HIV viral particles

i) **Reverse transcription**: Reverse transcriptase helps to produce a double stranded DNA (dsDNA) from viral RNA that can be inserted into the host genome.

ii) **Integration**: The new genetic material of virus enters the nucleus of the CD4+ T cells and integrates itself into host genetic material with the help of integrase.

iii) **Transcription**: The virus uses host enzymes to produce viral RNA and polypeptides.

iv) **Assembly**: An enzyme called protease helps in the processing of newly translated polypeptides into proteins. A new virus is assembled from the newly produced viral RNA and proteins.

v) **Budding**: This is the final stage of the HIV life cycle. In this stage, the newly assembled virus pushes itself out of the host cell, taking with it part of the membrane of the cell. This outer part covers the virus and contains all the structures necessary to bind to a new CD4+ T cell and begin the process again.

**A1.2 GB virus C (GBV-C)**

GBV-C is a single-stranded, positive-sense RNA virus belonging to the *Flaviviridae* family. It is a member of the *Pegivirus* genus (Stapleton et al., 2011). GBV-C infection is common and is distributed worldwide. It establishes persistent infection without any clinical symptoms or disease in both immunocompromised and healthy individuals. As the virus is non-pathogenic, affected individuals are not excluded from the donation of blood. In developed countries, one to four percent and in developing countries, up to twenty percent, of healthy blood donors are viremic with GBV-C at the time of blood donation (Mohr & Stapleton et al, 2009). GBV-C encodes two structural proteins namely, envelope glycoproteins E1 and E2 and six nonstructural proteins (NS) namely, NS2, NS3, NS4A, NS4B, NS5A and NS5B. GBV-C envelope proteins and nonstructural proteins are reported to modulate HIV infection.
A1.3 Inhibition of HIV in Presence of GBV-C

Studies reported that HIV-1 infected individuals who are co-infected with GBV-C survive longer than those without GBV-C, indicating inhibition of HIV-1 by GBV-C (Xiang et al., 2001; Polgreen et al., 2003; Williams et al., 2004; Zhang et al., 2006a; Shankar et al., 2011). Several mechanisms of HIV-1 inhibition by GBV-C are suggested. Table A1.1 is an attempt to summarize these mechanisms and the information in this table is compiled from the thesis of Mohr (2012).

Table A1.1: Mechanisms of HIV inhibition by GBV-C

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Mechanisms of Co-infected HIV inhibition by GBV-C</th>
<th>References</th>
</tr>
</thead>
</table>
| GBV-C infection *in vivo*        | ▪ Decreased expression of CCR5, CXCR4 on CD4+ T cells.  
                                   ▪ Decreased expression of CD38 on CD8+ T and CD4+ T cells.  
                                   ▪ Increased levels of interferon in dendritic cells.  
                                   ▪ Increased level of RANTES, MIP1α, MIP1β and SDF-1 produced by CD8+ T cells | Nattermann et al., 2003; Schwarze-Zander et al., 2010  
                                   Maidana-Giret et al., 2009  
                                   Rowland-Jones, 1999  
                                   Lalle et al., 2008 |
| GBV-C infection *in vitro*       | ▪ Decreased expression of CCR5, CXCR4 on PBMCs.  
                                   ▪ Increased level of RANTES, MIP1α, MIP1β and SDF-1 produced by PBMCs | Jung et al., 2005  
                                   Xiang et al., 2001  
                                   Xiang et al., 2004  
                                   Xiang et al., 2005b  
                                   Nattermann et al., 2003 |
| GBV-C NS5A protein               | ▪ Down regulates CXCR4 expression on CD4+ T cells.  
                                   ▪ Induces expression of SDF-1 in a CD4+ T.  
                                   ▪ 16 amino acid domain in NS5A inhibits HIV infection. | Chang et al., 2007  
                                   Xiang et al., 2005a  
                                   Xiang et al., 2007 |
| GBV-C E2 protein                 | ▪ Inhibits HIV replication and HIV env pseudotyped particle transduction in PBMCs.  
                                   ▪ Inhibits HIV env pseudotyped particle transduction in a dose dependent manner in human osteosarcoma cell line. | Jung et al., 2007  
                                   Mohr et al., 2012 |
| GBV-C E2 peptides                | E2 fusion peptide (269-286) inhibits HIVgp41 fusion.  
                                   E2 peptide (133-150) inhibits gp41-induced fusion in a cell-based fusion assay. | Herrera et al., 2009  
                                   Herrera et al., 2010 |
| GBV-C E2 antibodies              | Interacts with cellular proteins carried on the virus particle and inhibits HIV virion attachment to cells. | Mohr et al., 2010 |
The various mechanisms suggested above for the inhibition of HIV-1 by GBV-C assumes that the same cell is infected by both HIV-1 and GBV-C. Note that some cells will be infected only by HIV-1, while others only by GBV-C and a few cells will be co-infected by both HIV-1 and GBV-C. Remaining cells will be normal/uninfected. The Acquired Immunodeficiency Syndrome (AIDS) progresses as the number of cells infected by HIV-1 increases. Therefore, it is important to understand and predict the impact of GBV-C co-infection on cells infected by HIV-1. For this purpose, mathematical models were developed to understand only HIV-1 infection, only GBV-C infection and co-infection of HIV-1 and GBV-C. For these simulations, several assumptions were made not only to simplify the model but because of the lack of experimental data, particularly for GBV-C infection and co-infection. At the outset, it is important to state that because of these assumptions some of the simulation results are not close to reality but are only indicative in nature. These simulation results under the assumptions made are discussed below. Assumptions for the mathematical model are:

i. An individual suffers from no other infection except HIV-1 and/or GBV-C depending on the mathematical model under consideration.

ii. CD4+ T cells at the start of the simulations are uninfected by both HIV-1 and GBV-C in all the three models.

iii. Rate of production (λ) and rate of clearance (δ) of uninfected cells are assumed to be constant though unequal.

iv. Infection rate (b), production rate (k) and clearance rate (u) of HIV-1 and GBV-C are assumed to be constant though unequal.

The parameters for uninfected CD4+ T cells used to carry out simulations are summarized below in Table A1.2.
Table A1.2: Parameters for uninfected CD4+ T cells

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Parameter</th>
<th>Symbol in HIV infection, GBV-C infection and Co-infection model</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total CD4+ T cells in blood</td>
<td>Not applicable</td>
<td>5 x 10^9 cells</td>
<td>Ganusov &amp; De Boer, 2007</td>
</tr>
<tr>
<td>2</td>
<td>Average blood volume of an adult</td>
<td>Not applicable</td>
<td>6000 mL</td>
<td>Tsiang &amp; Gibbs, 2004</td>
</tr>
<tr>
<td>3</td>
<td>Activated CD4+ T cells</td>
<td>Not applicable</td>
<td>Reported to be 0.243 of the total CD4+ T cells. 0.243 x 5 x 10^9 = 1.215 x 10^9 cells</td>
<td>Ribeiro et al., 2002</td>
</tr>
<tr>
<td>4</td>
<td>Total number of activated CD4+ T cells per ml when time = 0.</td>
<td>$x_o$</td>
<td>$\frac{(1.215 \times 10^9)}{6000} = 2.025 \times 10^5$ cells/ml</td>
<td>Deduced from 2 and 3 in Table A1.2</td>
</tr>
<tr>
<td>5</td>
<td>Rate of proliferation of activated CD4+ T cells</td>
<td>Not applicable</td>
<td>0.005 day^{-1}</td>
<td>Ribeiro et al., 2002</td>
</tr>
<tr>
<td>6</td>
<td>Rate of proliferation of activated CD4+ T cells/ml</td>
<td>$\lambda$</td>
<td>$0.005 \times 2.025 \times 10^5 = 4.166 \times 10^3$ cells/ml</td>
<td>Deduced from Sr.No 4 and 5 in Table A1.2</td>
</tr>
<tr>
<td>7</td>
<td>Constant death rate of uninfected activated CD4+ T cells</td>
<td>$\delta$</td>
<td>0.136 day^{-1}</td>
<td>Ribeiro et al., 2002</td>
</tr>
</tbody>
</table>

A2 Mathematical Model of HIV-1 Infection (Absence of Co-infection)

In order to model the progression of HIV-1 infection in the absence of GBV-C, a mathematical model reported by Nowak and Bangham (1996) to study the dynamics of HIV infection was used,

$$\frac{dv_1}{dt} = k_1 y_1(t) - u_1 v_1(t)$$  \hspace{1cm} -- (Equation A1.1)  
$$\frac{dy_1}{dt} = b_1 v_1(t)x(t) - \delta_1 y_1(t)$$  \hspace{1cm} -- (Equation A1.2)  
$$\frac{dx}{dt} = \lambda - \delta x(t) - b_1 v_1(t)x(t)$$  \hspace{1cm} -- (Equation A1.3)

The parameters used in these equations and their values are summarized in Table A1.3. Values for $\lambda$ and $\delta$ were taken from Table A1.2.
Table A1.3: Parameters specific to HIV-1 infection and their values

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Parameter</th>
<th>Symbol in HIV infection and Co-infection model</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Infection rate of HIV</td>
<td>$b_1$</td>
<td>$4 \times 10^{-6}$ ml copies$^{-1}$ day$^{-1}$</td>
<td>Callaway &amp; Perelson, 2002</td>
</tr>
<tr>
<td>2</td>
<td>Virion clearance rate of HIV</td>
<td>$u_1$</td>
<td>13 day$^{-1}$</td>
<td>Callaway &amp; Perelson, 2002</td>
</tr>
<tr>
<td>3</td>
<td>Death rate of HIV infected CD4+ T cells</td>
<td>$\delta_1$</td>
<td>0.7 day$^{-1}$</td>
<td>Callaway &amp; Perelson, 2002</td>
</tr>
<tr>
<td>4</td>
<td>Viral production rate of HIV</td>
<td>$k_1$</td>
<td>100 copies cell$^{-1}$ day$^{-1}$</td>
<td>Adams et al., 2005</td>
</tr>
</tbody>
</table>

As can be seen, the Equations A1.1 to A1.3 are interdependent and hence they were used simultaneously to simulate the HIV-1 infection using MATLAB.

The initial values given as input to the model were

- Viral load: 400 copies/ml.
- CD4+ T cells ($x_0$): $2.025 \times 10^5$ cells/ml. (See Table A1.2)

The results obtained at the end of simulations for 100 days are given in Figure A1.1.

![Figure A1.1: Dynamics of uninfected CD4+ T cells, HIV-1 infected CD4+ T cells and HIV-1 viral load from HIV infection model](image)

A sharp increase in HIV-1 viral load up to six days is accompanied with an equivalent increase in HIV infected CD4+ T cells and decrease in uninfected cells (See Figure A1.1). After forty days the number of uninfected/HIV infected CD4+ T cells and HIV viral load remain constant. However, it is known that as AIDS progresses in an individual, HIV-1 infected cells and HIV-1 viral load increase rapidly. The unusual behaviour of HIV-1 infection model can be because of the assumption that HIV-1 has a
constant infection rate $b_1$. More realistic simulations can be undertaken in future with the help of refined model that incorporates time-dependent infection rate.

A3 Mathematical Model of GBV-C Infection

The experimental data published on GBV-C, unlike HIV-1, is very limited. The values for GBV-C clearance rate ($u_2$), infection rate ($b_2$) and production rate ($k_4$) were interpolated from available experimental data and are described in succeeding sections. The estimates may not be entirely realistic as the interpolations were based on certain assumptions and few experimental points; however, future experimental data is expected to provide more accurate parameter values.

A3.1 Parameter Estimation for GBV-C infection

A3.1.1 Virion Clearance Rate of GBV-C ($u_2$)

Information on time-dependent decrease in viral load from which clearance rate of GBV-C could be deduced was not available in published literature. Therefore, Prof. Jack Stapleton (Director, HIV Program, University of Iowa, whose laboratory is working on understanding the mode of co-infection of GBV-C and HIV as well as effect of co-infection of these viruses) was contacted to get information on decrease in GBV-C viral load with time. He was kind enough to provide unpublished experimental data on GBV-C viral load over a period of eighteen hours in six patients. Out of the six patients, only two patients were able to clear GBV-C viremia. Hence, viral load profile from only these two patients was subjected to non-linear regression analysis using the function $V(t) = v_0 * e^{-u_2*t}$ (viral decay function from Fischer et al., 2004). The value of $u_2$ from this analysis was 3.58 day$^{-1}$. However, as the experimental results did not show any specific trend, the best fitted curve had very low coefficient of determination. In the absence of any other experimental data, the value obtained for $u_2$ from this analysis was used in further simulations.

The GBV-C virion clearance rate $u_2$ deduced from above curve fitting was

$$u_2 = 3.58 \text{ day}^{-1}$$
A3.1.2 Infection Rate of GBV-C ($b_2$)

At steady state, the rate of clearance of infected cells is equal to the rate at which infected cells are produced (Coffin, 1995). Therefore, as per the Nowak and Bangham (1996) basic model of viral dynamics as applied to GBV-C dynamics, the rate of change of GBV-C infected cells can be given by

\[
dy_2/dt = b_2 u_2 x_2(t) - \delta_2 y_2(t) \quad ---- (Equation A1.4)
\]

At steady state, \(dy_2/dt = 0\)

\[.
\therefore b_2 u_2 x_{ss} = \delta_2 y_{ss}
\]

\[.
\therefore b_2 = (\delta_2 y_{ss})/(u_2 x_{ss}) \quad ---- (Equation A1.5)
\]

Here,

\(u_2\) = Virion clearance rate of GBV-C = 3.58 day\(^{-1}\) (From section A3.1.1)

\(x_{ss}\) = Activated uninfected CD4+ T cells at steady state.

\(y_{ss}\) = GBV-C infected cells at steady state.

The fraction of activated CD4+ T cells at steady state was reported to be 0.05 of the CD4+ T cell count in blood (Ribeiro et al., 2002). Estimated CD4+ T cell count in blood is equal to 5 x 10\(^9\). Considering that the average blood volume of an adult is equal to 6000 mL.

\[x_{ss} = (0.05 \times 5 \times 10^9)/6000\]

\[x_{ss} = 4.166 \times 10^4\]

\(\delta_2\) = Death rate of GBV-C infected CD4+ T cells was assumed to be same as \(\delta\) (see Sr.No 7 in Table A1.2)

\[.
\therefore \delta_2 = 0.136
\]

As reported in (Xiang et al., 2000), during GBV-C infection 20% of CD4+ T cells were infected with the virus. At steady state, the GBV-C infected cells will be

\[.
\therefore y_{ss} = 0.2 \times x_{ss} \quad ---- (Equation A1.6)
\]

\[y_{ss} = 0.2 \times 4.166 \times 10^4\]

\[y_{ss} = 8.332 \times 10^3\]

Substituting the above values in equation A1.5, the infection rate of GBV-C was predicted to be

\[b_2 = (0.136 \times 8.332 \times 10^3)/(3.58 \times 4.166 \times 10^4)\]

\[b_2 = 7.59 \times 10^{-3} \text{ ml copy}^{-1} \text{ day}^{-1}\]
A3.1.3 Production Rate of GBV-C from GBV-C Infected CD4+ T cells ($k_4$)

At steady state, viral production is equal to viral decay (Coffin, 1995). From basic model of virus (Nowak & Bangham, 1996)

\[ k_4 = \frac{u_2 v_{ss}}{y_{ss}} \]

--- (Equation A1.7)

$v_{ss}$ is viral load at steady state and can be estimated from copy number of virus per cell. GBV-C RNA was reported to be 0.15-0.2% of CD4+ T cells (George et al., 2003). Using the mean of these values, GBV-C RNA will be 0.175% of CD4+ T cells. Hence at steady state,

\[ v_{ss} = \frac{(0.00175 \times 5 \times 10^9)}{6000} = 1.4583 \times 10^3 \]

Viral load was estimated to be \( 1.4583 \times 10^3 \). This value of \( v_{ss} \) was used for further calculations. The value of \( u_2 = 3.58 \) day\(^{-1}\) (taken from section A3.1.1) and the value of \( y_{ss} \) (taken from Equation A1.6).

Therefore, \( k_4 \) will have value:

\[ k_4 = \frac{(3.58 \times 1.4583 \times 10^3)}{(8.332 \times 10^3)} = 0.62 \]

\[ k_4 = 0.62 \text{ copies cell}^{-1} \text{day}^{-1} \]

Table A1.4 Parameters specific to GBV-C infection and their values

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Parameter</th>
<th>Symbol in GBV-C infection and Co-infection model</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Production rate of GBV-C from GBV-C infected CD4+ T cells</td>
<td>$k_4$</td>
<td>0.62 copies cell(^{-1}) day(^{-1})</td>
<td>Explained in section A3.1.3</td>
</tr>
<tr>
<td>2</td>
<td>Virion clearance rate of GBV-C</td>
<td>$u_2$</td>
<td>3.58 day(^{-1})</td>
<td>Explained in section A3.1.1</td>
</tr>
<tr>
<td>3</td>
<td>Infection rate of GBV-C virion</td>
<td>$b_2$</td>
<td>$7.59 \times 10^3$ ml copy(^{-1}) day(^{-1})</td>
<td>Explained in section A3.1.2</td>
</tr>
<tr>
<td>4</td>
<td>Death rate of GBV-C infected CD4+ T cells</td>
<td>$\delta_2$</td>
<td>0.136 day(^{-1})</td>
<td>As GBV-C does not appear to cause infection, value of $\delta_2$ is assumed to be same as $\delta$</td>
</tr>
</tbody>
</table>

A basic mathematical model of viral dynamics reported by Nowak and Bangham (1996) was used to study the progression of GBV-C infection in absence of HIV-1. The equations to model the GBV-C dynamics are

\[ \frac{dv_2}{dt} = k_2 y_2(t) - u_2 v_2(t) \]

--- (Equation A1.8)

\[ \frac{dy_2}{dt} = b_2 v_2(t) x(t) - \delta_2 y_2(t) \]

--- (Equation A1.9)
\[ \frac{dx}{dt} = \lambda - \delta x(t) - b_2 v_2(t)x(t) \]  

\[ \text{Equation A1.10} \]

The parameter definitions and values used are given in Table A1.2 and Table A1.4. The simulations were performed in MATLAB and the equations A1.8 to A1.10 were solved simultaneously as they are interdependent.

The initial values given as input to the model were
- Viral load: 400 copies/ml.
- CD4+ T cells \( (x_0) \): \( 2.025 \times 10^6 \) cells/ml. (See Table A1.2)

The results obtained at the end of simulations for 100 days are given in Figure A1.2.

![Figure A1.2: Dynamics of uninfected CD4+ T cells, GBV-C infected CD4+ T cells and GBV-C viral load from GBV-C infection model](image)

As can be seen from Figure A1.2, GBV-C infects large number of CD4+ T cells during first day of simulations itself. A comparison of Figure A1.1 and A1.2 shows that GBV-C establishes infection much faster than HIV-1. Even in this case, the constant number of uninfected/infected CD4+ T cells and constant viral load can be an artefact of the assumptions made to predict model parameters. The model can be improved with availability of experimental data for GBV-C.
A mathematical model representing the co-infection of HIV-1 and GBV-C was developed to understand the impact of GBV-C infection on progression of HIV-1 infection. Figure A1.3 gives a pictorial representation of co-infection viral dynamics of HIV-1 and GBV-C.

In addition to the assumption listed in section A1.3, the assumptions specific to co-infection model are

1. HIV-1 and GBV-C have equal chance of infecting an uninfected cell.
2. At initial stage the number of uninfected cells is sufficiently large so that there will be no competition between HIV-1 and GBV-C for the same CD4+ T cell.
3. In co-infection model, both the viruses can infect the same cell as their mechanism for entry and rate of infection differs from each other to some extent.

The parameters specific to co-infection of GBV-C and HIV-1 infection are summarized in Table A1.5.
Table A1.5 Parameters specific to co-infection and their values

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Parameter</th>
<th>Symbol in co-infection model</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Death rate of co-infected CD4+ T cells</td>
<td>$\delta_3$</td>
<td>$\delta_3 = (0.7 + 0.136)/2 = 0.418 \text{ day}^{-1}$</td>
<td>Death rate of co-infected cells was assumed to be equal to the mean of $\delta_1$ and $\delta_2$.</td>
</tr>
<tr>
<td>2</td>
<td>Production rate of GBV-C from co-infected CD4+ T cells</td>
<td>$k_2$</td>
<td>0.62 copies cell $\text{day}^{-1}$</td>
<td>Assuming that there is no inhibition of GBV-C by HIV in co-infected cells, value of $k_2$ was taken to be equal to $k_4$ (see Sr.No 1 in Table A1.4)</td>
</tr>
<tr>
<td>3</td>
<td>Production rate of HIV from co-infected CD4+ T cells</td>
<td>$k_3$</td>
<td>$k_3 = 0.560 \times k_1 = 0.560 \times 100$</td>
<td>Xiang et al. (2001) reported that production rate of HIV from co-infected cells ($k_3$) will be 50.6% of production rate of HIV-1 from HIV infected cells ($k_1$). $k_3 = 100$ copies cell$^{-1}$ day$^{-1}$ (See Sr.No 4 in Table A1.3)</td>
</tr>
</tbody>
</table>

The co-infection model of HIV-1 and GBV-C was expressed in terms of six differential equations, modelling the changes in uninfected cells $x$, infected cells $y_i$ ($i = 1, 2, 3$) and viral load $v_j$ ($j = 1, 2$)

i. Rate of change in HIV-1 viral load
   \[
   \frac{dv_1}{dt} = k_1 y_1(t) + k_3 y_3(t) - u_1 v_1(t) \quad \text{---- (Equation A1.11)}
   \]

ii. Rate of change in GBV-C viral load
   \[
   \frac{dv_2}{dt} = k_2 y_3(t) + k_4 y_2(t) - u_2 v_2(t) \quad \text{---- (Equation A1.12)}
   \]

iii. Rate of change of HIV-1 infected cells
    \[
    \frac{dy_1}{dt} = b_1 v_1(t) x(t) - \delta_1 y_1(t) - b_2 v_2(t) y_1(t) \quad \text{---- (Equation A1.13)}
    \]

iv. Rate of change of GBV-C infected cells
    \[
    \frac{dy_2}{dt} = b_2 v_2(t) x(t) - \delta_2 y_2(t) - b_1 v_1(t) y_2(t) \quad \text{---- (Equation A1.14)}
    \]

v. Rate of change of co-infected cells
   \[
   \frac{dy_3}{dt} = b_2 v_2(t) y_1(t) + b_1 v_1(t) y_2(t) - \delta_3 y_3(t) \quad \text{---- (Equation A1.15)}
   \]

vi. Rate of change of uninfected cells
    \[
    \frac{dx}{dt} = \lambda - (\delta + b_1 v_1(t) + b_2 v_2(t)) x(t) \quad \text{---- (Equation A1.16)}
    \]

The parameters pertaining to CD4+ T cells, HIV-1 infection, GBV-C infection and co-infection were taken respectively from Tables A1.2, A1.3, A1.4 and A1.5.
equations from A1.11 to A1.16 are interdependent and hence were used simultaneously to simulate the co-infection of HIV-1 and GBV-C using MATLAB.

The initial values given as input to the model were

- Viral load: 400 copies/ml.
- CD4+ T cells ($x_0$): $2.025 \times 10^5$ cells/ml. (See Table A1.2)

The results at the end of simulations for 100 days for the uninfected, HIV-1 infected CD4+ T cells and GBV-C infected CD4+ T cells from the co-infection model are given in Figure A1.4.

As can be seen from Figure A1.4, the HIV-1 infected CD4+ T cells are much lower than the GBV-C infected CD4+ T cells, indicating inhibition of HIV-1 infection in the presence of GBV-C. As expected, it also shows a proportional decrease in the uninfected CD4+ T cell count. As mentioned earlier, the constant number of uninfected and infected CD4+ T cells, after around forty days of simulations, can be an artefact of assumptions made to carry out simulations. This co-infection model can be used as a base model to build more sophisticated models as and when experimental data becomes available.

The results from the co-infection model were compared with the results from HIV-1 infection model (section A2). These comparisons are discussed in succeeding sections.
A4.1 Dynamics of CD4+ T Cells that are not Infected with HIV-1 from HIV-1 Infection Model and Co-infection Model

As can be seen from Figure A1.5, the uninfected cells in HIV infection model (blue curve) decrease rapidly as compared to the HIV-1 uninfected cells in co-infection model (green curve). This observation suggests that there is a delay in the infection of uninfected CD4+ T cells by HIV-1 in presence of the GBV-C. It can be seen from the Figure A1.5 that the uninfected CD4+ T cell count from co-infection model is slightly higher than that of HIV-1 infection model. Although this difference is not very significant, it suggests that the co-infection model shows the property of inhibiting HIV-1 infection.

A4.2 Dynamics of HIV-1 Infected CD4+ T cells from HIV-1 Infection Model and Co-infection Model

As can be seen from Figure A1.5, the uninfected cells in HIV infection model (blue curve) decrease rapidly as compared to the HIV-1 uninfected cells in co-infection model (green curve). This observation suggests that there is a delay in the infection of uninfected CD4+ T cells by HIV-1 in presence of the GBV-C. It can be seen from the Figure A1.5 that the uninfected CD4+ T cell count from co-infection model is slightly higher than that of HIV-1 infection model. Although this difference is not very significant, it suggests that the co-infection model shows the property of inhibiting HIV-1 infection.
As can be seen from Figure A1.6, the number of HIV-1 infected CD4+ T cells from the co-infection model is lower than the number of HIV-1 infected cells from the HIV infection model. The Figure A1.6 also shows the absence of HIV-1 infected cells for a short duration (around six to fifteen days) in the co-infection model. The reason for such behaviour can be attributed to the lack of uninfected CD4+ T cells in the co-infection model, as the CD4+ T cells are rapidly infected with GBV-C. The progression of HIV-1 infection in case of co-infection with GBV-C is reported to be very different and hence the co-infected CD4+ T cells were not compared with HIV-1 infected CD4+ T cells. It was also observed that the HIV-1 viral load in co-infection model is lower as compared to that in HIV infection model suggesting inhibition of HIV-1 by GBV-C.

A5 Conclusions
The co-infection model of GBV-C and HIV-1 is based on many assumptions. However, the simulation results shows lower HIV-1 infected cells and lower HIV-1 viral load in the presence of GBV-C as against its absence. The co-infection model presented in this study thus gives an idea about the relative changes in HIV-1 infection and suggests delay in the progression of HIV-1 infection. It is understood that the model is not close to reality because of the assumptions mentioned above, but this opens up a new therapeutic strategy of utilizing an anti-virus against a viral infection. Very little understanding exists at this stage to be able to identify pairs of virus-anti-virus. Thus there is a need to determine common features between such pairs. It is becoming clear that the virus and anti-virus should have i) same genetic material DNA/RNA and ii) should bind to the same cells and their mechanism of entry and integration should be similar. A good virus-anti-virus pair should use the same binding site on the cell but may also have independent binding sites for the virus and anti-virus affecting the properties of the cell. The rate of growth and rate of replication for the virus and anti-virus should be similar. Further, to acquire prolonged immunity from the virus, strategies for virus clearance should be developed carefully so as to allow growth and replication of anti-viruses. In short, the anti-virus can be used to treat the infection caused by the virus as well as to create immunity against the infectious virus as virus databases containing various properties and modes of infection of viruses are becoming readily available.