Quantifying the infectivity of human immunodeficiency virus
(viral infectivity assay/human immunodeficiency virus kinetics/gp120 blocking protein)

Scott P. Layne*, John L. Spouge†, and Micah Dembo*

*Theoretical Division, Los Alamos National Laboratory, Los Alamos, NM 87545; and †Laboratory of Mathematical Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

ABSTRACT We have developed a mathematical model that quantifies lymphocyte infection by human immunodeficiency virus (HIV) and lymphocyte protection by blocking agents such as soluble CD4. We use this model to suggest standardized parameters for quantifying viral infectivity and to suggest techniques for calculating these parameters from well-mixed infectivity assays. We discuss the implications of the model for our understanding of the infectious process and virulence of HIV in vivo.

Subsets of lymphocytes, monocytes, and macrophages expressing CD4 are the primary targets for infection by human immunodeficiency virus (HIV) (1), and three overall steps have been suggested for the infective process. First, HIV diffuses to the cell surface; second, gp120 (120-kDa glycoprotein) on the virus' surface and CD4 on the target cell's surface form a bimolecular complex; third, interactions involving CD4, gp120, and gp41 promote fusion of HIV envelope with target cell membrane, resulting in entry of the viral core (2–4). Given this mechanism, blocking some or all gp120 molecules on the viral surface should inhibit infection (5), and, consequently, soluble forms of CD4 (sCD4) have been suggested as potential therapeutic agents. In fact, several studies have demonstrated that sCD4 blocks HIV infection in vitro (6–10).

In this paper, we develop a mathematical model quantifying the kinetics of target cell infection by HIV and target cell protection by sCD4. The model is concerned with infection from the fluid phase and does not address direct cell-to-cell transmission—e.g., syncytia formation (11). We show how the model can be used to analyze the results of well-mixed viral infectivity assays and to determine parameters that influence the initial steps in infection. The model also has implications for our understanding of the infectious process and virulence of HIV in vivo and on the prospects for therapy with sCD4.

THE MODEL

Consider a stock solution prepared from the supernatant of a cell culture infected with a particular strain of HIV. Such a stock solution can be regarded as a mixture of "homogeneous cohorts" of virions (i.e., populations of virions that were born simultaneously and that have been treated identically ever since). At birth, all members of a homogeneous cohort are assumed to be identical. A virion is said to remain "live" at time T if it has neither participated in an infective event nor been nonspecifically killed. As time progresses, some cohort members will die and the "infectivity" of these remaining live will diverge due to random processes.

Now suppose that V₀ random members of a homogeneous cohort are selected at birth. These virions are allowed to preincubate for time Tₚ and are then inoculated at T = 0 into a chamber containing a large excess of CD4⁺ target cells (see Fig. 1A). The objective of this procedure is to count the number of virions that successfully infect, I, which then yields the probability that a single virion will successfully infect, i = I/V₀. Considering each homogeneous cohort separately involves no loss of generality, since the behavior of a mixture of cohorts is obtained by taking a weighted average.

Fig. 1B illustrates the random processes acting on a cohort. Of these, blocking, shedding, and infection depend on gp120; nonspecific killing does not. The "equivalent site approximation" from polymer chemistry gives a manageable formulation of Fig. 1B with a minimal loss of detail (15–17). According to this approximation, each gp120 molecule on the surface of a "live" virion has the same chance of being shed, of binding to CD4 on a target cell, or of binding to sCD4 in solution (i.e., each gp120 has the same cross section for reaction). Furthermore, nonspecific killing operates independently on each live virion, regardless of its number of gp120 molecules.

Let N be the initial number of gp120 molecules on each virion at birth and let g be the probability that a particular gp120 remains at a later time. Since gp120s can be either free or complexed with sCD4, g = (F + C)/NV, where F and C are the numbers of free and complexed gp120 molecules on live virions and V is the number of live virions. Because of the equivalent site approximation, the probability that a live virion's surface will present exactly J gp120s is always given by a binomial distribution:

\[ P(J) = \binom{N}{J} g^J (1 - g)^{N-J}. \]  

Since F/NV is the probability that a given gp120 on a live virion is free and C/NV is the probability that a given gp120 on a live virion is complexed, it follows that each infective event causes the loss (on average) of \([1 + (N - 1)F/NV]\) free gp120 molecules and \([N - 1]C/\) complexed gp120 molecules.

Now let L and B be the respective concentrations of target cells and sCD4 in the reaction chamber. Because the viral inoculum is small, both L and B remain unperturbed and the kinetics in the reaction chamber are governed by:

\[ \frac{dI}{dT} = k_iLF, \]  
\[ \frac{dV}{dT} = -k_iLF - k_nV, \]  
\[ \frac{dF}{dT} = -k_iBF + k_C - (k_s + k_k)F \]  
\[ -k_iLF \left[ 1 + \frac{F}{NV} \right], \]  
\[ \frac{dC}{dT} = k_iBF - k_C - (k_s + k_k)C - k_iLF \left[ \frac{(N - 1)C}{NV} \right]. \]

Fig. 1B defines the five rate constants: kᵢ, kₙ, kₖ, and kₖ. The terms kᵢLF and kₙV are the rates of loss of live viral

Abbreviations: HIV, human immunodeficiency virus; sCD4, soluble CD4 protein.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.
particles due to infection and nonspecific killing, respectively. The terms $k_0B$ and $k_0C$ are the rates of formation and disassociation of gp120–sCD4 complexes, respectively. The terms $(k_i + k_k)F$ and $(k_i + k_k)C$ are the respective rates of loss of free and complexed gp120 from live virions due to the combined effects of spontaneous shedding and nonspecific killing of virus. Finally, the terms $k_iL[F(N - 1)F/NV]$ and $k_iL[F(N - 1)/C/NV]$ are the respective rates of loss of free and complexed gp120 from live virions due to infective events.

The initial conditions for Eqs. 2–5 depend on circumstances during the preincubation phase. In most assays, virions have no opportunity to infect target cells during preincubation ($L = 0$) and are not exposed to sCD4 ($B = 0$). The conditions at $T = 0$ are then: $f = 0$, $V = V_0 \exp(-k_aT_p)$, $F = NV_0 \exp(-(k_a + k_0)T_p)$, and $c = 0$.

To facilitate analysis, introduce nondimensional variables $i = I/V_0$, $v = V/V_0$, $f = F/NV$, and $c = C/NV$. Also introduce nondimensional time, $t = (k_i + k_k)T$, and nondimensional parameters $\gamma = k_i/(k_i + k_k)$, $\lambda = k_iL/(k_i + k_k)$, $\gamma = k_i/(k_i + k_k)$, and $\beta = k_B/k_i$. Then Eqs. 2–5 take the form:

$$\frac{di}{dt} = N\alphafv,$$
$$\frac{dv}{dt} = -[N\alpha f + 1 - \alpha]\nu,$$
$$\frac{df}{dt} = -\gamma(\beta f - c) - \sigmaf - \alpha(k_f - f),$$
$$\frac{dc}{dt} = \gamma(\beta f - c) - \sigmac + \lambda fc.$$

The quantity $i$ is the probability that an infectious virion born at $-t_p$ infects by time $t$. This is the main quantity of biological interest derived from infectivity assays.

**Parameter Estimation.** T lymphocytes used in viral infectivity assays typically display $r = 2 \times 10^4$ CD4 receptors (18). According to Berg and Purcell (19), the probability that a viral gp120 diffusing to a lymphocyte will find a CD4 receptor is $R_{R_b}/(R_{R_b} + \pi R_l) = 0.8$ (Table 1). Electron microscope studies (20–22) estimate that 70–80 gp120 complexes cover a mature virion (a single gp120 complex covers $1/100th$ of a virion’s surface). Therefore, we take $N = 80$ and the Smoluchowski formula for diffusional collision between two spherical particles (23) gives the rate constant for infection: $k_i = (0.8)(0.01)4\pi R_l(R_b + R_l) = 8.0 \times 10^{-12}$ cm$^3$ sec$^{-1}$. Experiments with other viruses indicate that $k_i$ is unlikely to be more than 1000 times smaller than this upper limit (24).

The Smoluchowski equation along with estimates from Table 1 also yield an upper limit for the forward rate constant of the blocking reaction: $k_b = 4\pi(D_c + D_t)(R_c + R_t) = 3.0 \times 10^{-12}$ cm$^3$sec$^{-1}$. Again, we would not expect $k_b$ to be more than 1000 times smaller than this upper limit.

The association constant between sCD4 and gp120 (6, 25) ranges from 0.25 to $10^9$ to $1.4 \times 10^9$ M$^{-1}$. Accordingly, we take $k_{asso} = k_i/k_b = 1.2 \times 10^8$ M$^{-1}$ = $2.0 \times 10^{-12}$ cm$^3$ molecule$^{-1}$. Given the fixed ratio $k_{asso}$, the reverse rate constant for the blocking reaction is $k_r = k_f/k_{asso} = 1.5$ sec$^{-1}$. HIV-1 strains III B and RFII lose half of their infectivity in 4–6 hr at 37°C [P. L. Nara and J. Kessler, personal communication (using the assay in ref. 26)]. This gives $k_b + k_k = 10^{-4}$ sec$^{-1}$ molecule$^{-1}$ to within a factor of 2. Unless specified, we use the above parameters for all numerical calculations.

**Numerical Solutions.** Fig. 2 illustrates numerical solutions of the model for typical parameters: Fig. 2A shows a case with no blocker; Fig. 2B shows the effect of adding a low concentration of blocker; Fig. 2C shows the effect of adding a higher concentration of blocker; and Fig. 2D shows the effect of a high concentration of blocker in conjunction with nonspecific killing—e.g., by nonoxynol-9 (27).

![Fig. 1.](image) The three phases in a viral infectivity assay. Virions are born at $T = T_p$. During the preincubation phase, $T < T_p$, shedding and nonspecific killing occur but target cell infection does not occur since $L = 0$. During the incubation phase, $0 < T < 0.1$, all three processes of shedding, nonspecific killing, and target cell infection occur. At $T > 10^5$ sec, new virions start emerging from infected cells and secondary infections occur. (B) The kinetic processes in a viral infectivity assay. $k_f$ is the rate constant for successful infective contact between viral gp120 and CD4 on a target cell, defined on a per gp120 basis. When a virion sheds all of its gp120, it is considered live but not infectious. $k_n$ is the rate constant for nonspecific killing of virions, which includes mechanisms such as enzymatic degradation, dissociation by soaps, and neutralization by lipoprotein vesicles (12). Complement does not appear to contribute to nonspecific killing of HIV (13). The processes of infection and nonspecific killing result in the disappearance of virions together with their associated free and complexed gp120s. $k_t$ is the rate constant for spontaneous disassociation of gp120 from gp41 (14). Although such “shedding” causes progressive inactivation of virions, it does not cause the actual disappearance of virions. $k_t$ and $k_r$ are the forward and reverse rate constants for gp120–sCD4 complex formation, respectively. These processes result in the masking and unmasking of gp120s but do not result in the net loss of gp120 or in the disappearance of virions.

In Fig. 2A, the number of infected target cells rises linearly until $T = 1 \times 10^5$ sec. Subsequently, at the characteristic shedding time $k_s^{-1} = 1 \times 10^5$ sec, there is a drop in the number of gp120 molecules on the surface of virions and the rate of target cell infection diminishes. The obvious decline in the number of virions at $T \approx 1 \times 10^5$ sec is due to target cell infection. When target cell infection stops, $T = 1 \times 10^5$ sec, 72% of initial virions have infected target cells; the remaining 28%, now completely lacking gp120 molecules and hence noninfectious, remain in the media. In this computation, there is no nonspecific killing and, at least in theory, these live but noninfectious particles remain in solution indefinitely.

Fig. 2B shows the effects of adding a small concentration of sCD4 to the culture medium. The initial rate of target cell infection is unchanged from Fig. 2A until viral gp120 and sCD4 begin to equilibrate at $T = 1 \times 10^{-1}$ sec. Immediately

**Table 1. Summary of diffusion rates**

<table>
<thead>
<tr>
<th>Object</th>
<th>Radius, cm</th>
<th>Diffusion rate, cm$^2$sec$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV virion (20–22)</td>
<td>$R_e = 5.0 \times 10^{-6}$</td>
<td>$D_e = 2.0 \times 10^{-8}$</td>
</tr>
<tr>
<td>CD4* lymphocyte</td>
<td>$R_e = 4.0 \times 10^{-4}$</td>
<td>$D_e = 2.5 \times 10^{-10}$</td>
</tr>
<tr>
<td>gp120</td>
<td>$R_e = 3.3 \times 10^{-7}$</td>
<td>$D_e = 3.0 \times 10^{-7}$</td>
</tr>
<tr>
<td>sCD4 molecule (50 kDa)</td>
<td>$R_e = 2.5 \times 10^{-7}$</td>
<td>$D_e = 4.0 \times 10^{-7}$</td>
</tr>
</tbody>
</table>

The diffusion rate of a sphere of radius $R$ is approximately $kT/6\pi\eta R$, where $k$ is the Boltzmann constant, $T$ is temperature, and $\eta$ is viscosity (23). We use $\tau = 300$ K, $\eta \approx 2 \times 10^{-2}$ poise, and assume that protein occupies $1.23 A^3$ per Da.
following this, the rate of target cell infection declines by a factor of 2–3 and, because of this decline, only 35% of the initial virions ultimately find target lymphocytes, a decline comparable to the blocking ratio.

In Fig. 2C, the concentration of sCD4 is 100-fold higher than in Fig. 2B. Consequently, equilibration of the block with gp120 occurs in only $3 \times 10^{-3}$ sec and 199 of 200 gp120 molecules are blocked. The rate of infectious events declines by the same proportion but the gp120 shedding is unchanged. Therefore, the final proportion of infecting virions is only 0.6%.

Fig. 2D shows the synergy of a high concentration of sCD4 with nonspecific killing of virus. Let us presume a rate constant for nonspecific killing, $k_a = 5 \times 10^{-4}$ sec$^{-1}$, that is 5-fold faster than the rate constant for gp120 shedding, $k_s = 1 \times 10^{-4}$ sec$^{-1}$. As in Fig. 2C, binding of sCD4 to viral gp120 and the shedding of viral gp120 are independent of nonspecific killing and occur on a "per virion" basis. Nonspecific killing causes the disappearance of virions and so infection stops before virions shed all of their gp120 molecules. As a result, the final proportion of infecting virions is diminished by a factor of 6 relative to Fig. 2C.

**Analytical Solutions.** In Fig. 2, viral gp120 and sCD4 reach equilibrium rapidly compared to target cell infection, a property holding for the full range of physically relevant parameters. Consequently, the usual quasi steady-state approximation, $k_B F = k_C$, permits asymptotic solutions to Eqs. 6–9. This approximation holds for time scales longer than the gp120–sCD4 equilibrium time (derivation given in ref. 28).

Recall that $g = f + c$ is the nondimensional concentration of both free and complexed gp120 on live virions. Because of the quasi steady-state approximation, $c = \beta g/(1 + \beta)$ and $f = g/(1 + \beta)$. Adding Eqs. 8 and 9 and applying these relations gives the Bernoulli equation:

$$\frac{dg}{dt} = -\sigma g - \lambda(1 + \beta)^{-1}g(1 - g).$$  \hspace{1cm} [10]

The nondimensional form of the initial conditions at $t = 0$ is: $i_0 = 0$, $v_0 = \exp(-1 - \sigma)t_0$ and $g_0 = \exp(-\sigma t_0)$.

Eq. 10 is solvable by separation of variables, and integral forms of $v(t)$ and $i(t)$ are obtained as described elsewhere (28). When $N \gg 1$, these forms lead to a good estimate of the value of $i(t)$ as $t \to \infty$:

$$i_\infty = e^{-(1 - \sigma)t_0} \frac{\xi N}{N - 1} e^{-\zeta/8} \sum_{j=0}^{\infty} \frac{(\xi/8)^j}{(1 + \delta j)!},$$  \hspace{1cm} [11]

where $\zeta = \exp(-\sigma t_0)(N - 1)/(\lambda + 1 + \beta)$ and $\delta = (\lambda + \sigma(1 + \beta))/(\lambda + 1 + \beta)$. Notice that $\zeta \leq N - 1$ and $\delta \leq 1$. The parameter $\zeta$ is a measure of the degree to which assay conditions promote target cell infection. The expansions of Eq. 11 for both $\zeta \to 0$ and $\zeta \to N - 1$ lead to the expressions

$$I_\infty = \frac{N \nu_0 k_f L e^{-k_f t_s}}{k_s + (k_a + k_i)(1 + B k_{assoc})} \left\{ 1 - \frac{\xi}{1 + \delta} + O(\xi^2) \right\},$$  \hspace{1cm} [12]

and

$$I_\infty = \frac{N \nu_0 e^{-k_f t_s}}{N - 1} \left\{ 1 - \frac{1 - \delta}{\zeta} + O(\zeta^{-2}) \right\},$$  \hspace{1cm} [13]

respectively. Notice that $\delta$ appears only in the higher-order terms.

The main use of Eqs. 12 and 13 is for design and analysis of experiments to measure the viral parameters $k_{assoc}$, $k_s$, $k_n$, $k_i$, and $N \nu_0$. Fig. 3 illustrates the transition from the regime of Eq. 12 (small $\zeta$) to the regime of Eq. 13 (large $\zeta$).
Consider an experiment to determine \( k_{assoc} \). In such an experiment, \( I_{w} \) would be measured at various values of the blocker concentration, \( B \), with all other variables held constant. When target cell concentration \( L \) is moderate, Eq. 12 implies that a plot of \( I_{w}(B = 0)/I_{w}(B > 0) \) versus \( B \) will be linear with slope \( = k_{assoc}/(1 + \lambda) \) and intercept = 1. Fig. 4 shows five plots generated by numerical solutions of Eqs. 2–5 simulating such an experiment at different lymphocyte concentrations, \( L \). Although the plots for all values of \( L \) appear linear, the fact that \( \lambda = L \) means that the slopes seriously underestimate \( k_{assoc} \) except at the lowest cell concentrations. Hence, when determining \( k_{assoc} \) from the inhibition of viral infectivity, the experiment must be performed within the regime where the results are independent of cell concentration—i.e., \( \lambda << 1 \).

Measuring the decay of viral infectivity with increasing preincubation times allows estimation of \( k_{o} \) and \( k_{s} \). Fig. 5 shows five curves generated by numerical solution of Eqs. 2–5 simulating such experiments at different choices of non-specific killing, \( k_{s} \). Target cell concentration is made as large as possible and no blocker is added (\( B = 0 \)). Under these conditions, it can be shown (Eq. 13) that the initial decay rate gives \( k_{s} \) and that the final decay rate gives \( k_{o} + k_{s} \) (Eq. 12). The increase in decay rate with preincubation is a consequence of a fundamental kinetic difference between non-specific killing and shedding. The former is a so-called "single-hit" process, whereas the latter is a "multi-hit" process inactivating the virus via incremental steps (i.e., loosing a few gp120s makes little difference to the initial infection rate). The lumped quantity, \( k_{o} + k_{s} \), is a direct measure of the ability of a viral strain to survive until it finds a target cell. A change in either \( k_{o} \) or \( k_{s} \) provides an objective measure of the potencies of viricidal agents.

Conducting two "preincubation assays" as described above with different target cell concentrations yields estimates of both \( NV_{O} \) and \( k_{s} \) (Fig. 6). The quantity \( NV_{O} \) is useful for estimating the number of "infectious" virions in the inoculum. The rate constant \( k_{s} \) is important because it quantifies the susceptibility of a particular target cell type to infection by a particular HIV strain. A decrease in \( k_{s} \) can be caused by a number of independent factors—e.g., a decrease in the surface density of CD4, an increase in the viral uncoating and penetration time, or an increase in the abortive disassociation of the initial virus-target cell complex. Fisher et al. (29)

![Fig. 4](image)

**Fig. 4.** Numerical solutions simulating a series of infectivity assays for quantifying \( k_{assoc} \). Affinity is measured by comparing an assay without blocker to an assay with blocker, holding other conditions identical. This control/experiment ratio is expressed by \( I(B = 0)/I(B > 0) \). The five straight lines correspond to increasing concentrations of target cells: \( O \), \( L = 2 \times 10^{9} \); \( O \), \( 2 \times 10^{7} \); \( \bullet \), \( 2 \times 10^{6} \); \( \circ \), \( 2 \times 10^{5} \); \( \Delta \), \( 2 \times 10^{4} \). The corresponding slopes for these solutions are \( 2.0 \times 10^{-12}, 1.9 \times 10^{-12}, 1.1 \times 10^{-12}, 8.0 \times 10^{-14}, \) and \( 0 \) cm⁻² molecule⁻¹⁻¹, respectively. According to Eq. 12, these slopes provide estimates of the quantity \( k_{assoc}/(1 + k_{s}L/(k_{o} + k_{s})), \) which is the effective association constant between blocker and gp120. The decline of the slopes with increasing target cell concentration occurs because \( k_{s}L/(k_{o} + k_{s}) \) increases. For all solutions \( T_{p} = 0 \), \( k_{o} = 0 \), \( k_{s} = 1 \times 10^{-4} \) sec⁻¹, and the incubation time \( T = 6.48 \times 10^{6} \) sec.

![Fig. 5](image)

**Fig. 5.** Five numerical solutions of the model simulating a series of experiments to determine \( k_{o} \) and \( k_{s} \). In all five simulations, virions are allowed to preincubate for various times \( T_{p} \) and are then inoculated into a reaction chamber. The five plots correspond to increasing amounts of nonspecific killing: \( O \), \( k_{s} = 0 \); \( O \), \( 0.5 \times 10^{-4} \); \( \Delta \), \( 1.0 \times 10^{-4} \); \( \bullet \), \( 2 \times 10^{-4} \); \( \circ \), \( 4 \times 10^{-4} \) sec⁻¹. The ordinate is normalized by the initial number of virions, which is equivalent to taking \( V_{O} = 1 \) in Eqs. 12 and 13. Initially, the slope of each plot is \( k_{o} \) but at longer preincubation times, the slope increases and approaches \( k_{s} + k_{o} \). Based on Eq. 13, extrapolating the final slope to \( T_{p} = 0 \) (top curve) gives the intercept \( NV_{O}k_{s}L/(k_{s}L + k_{o} + k_{s}) \). For all solutions \( k_{o} = 1 \times 10^{-12} \) sec⁻¹, \( B = 0 \), \( L = 1 \times 10^{6} \) cm⁻³, and the incubation time \( T = 6.48 \times 10^{6} \) sec.

and Cheng-Mayer et al. (30) report that different HIV isolates vary markedly in their capacity to propagate in vitro. A numerical ranking of virus-target cell "tropism" according to the value of \( k_{s} \) would help to clarify whether such variations are due to increased transmission or increased production of virions.

**DISCUSSION**

Five publications report that sCD4 blocks HIV infection of CD4⁺ lymphocytes (6–10) but, of these, only two provide sufficient information to determine \( k_{assoc} \). From figure 4 of Deen and co-workers (9, 31) the ratio of infection between "delayed control" and "experiment" yields \( k_{assoc} = 3.4 \times 10^{-12} \) cm⁻³ molecule⁻¹⁻¹. Figure 3 of Hussey et al. (8) gives \( k_{assoc} \approx 3.8 \times 10^{-12} \) cm⁻³ molecule⁻¹⁻¹ for both sCD4 derivatives.

These results should be compared to values of \( k_{assoc} \) measured for different analogs of sCD4 using direct physical methods (6, 25): \( 0.42 \times 10^{-12} \leq k_{assoc} \leq 2.3 \times 10^{-12} \) cm⁻³ molecule⁻¹⁻¹. The fair agreement of \( k_{assoc} \) as determined by physical and biological methods, strongly supports the fundamental assumption that infection proceeds at a rate proportional to the number of unblocked gp120s on a virion's surface (i.e., the equivalent site approximation). In particu-
lar, this agreement would not ensue if infection from the fluid phase did not require gp120 or if blocking essentially all viral receptors were necessary to diminish infection. The fact that sCD4 inhibits viral infection despite long incubation times confirms the existence of spontaneous nonspecific killing or shedding processes, $k_n + k_s$, and the importance of such processes in limiting viral infection. If virions did not lose activity with time, then eventually all five virions would infect despite block.

The expression phase of an infectivity assay can be likened to a branching process. In this process, each primary infection generates (on average) $V_{n}$ secondary virions that enter the culture medium without preincubation ($T_P = 0$). These secondary virions, in turn, infect new target cells with probability $i_{n}$. A growing infection develops if the branching number (the average number of successfully infecting secondary virions) is $V_{n} > 1$.

Blocking secondary infection with sCD4 permits estimation of the branching number for an unblocked infection. Define $B_{min}$ as the minimum sCD4 concentration extinguishing the branching process. Under many circumstances (Eq. 12), it can be shown that $V_{n} = N V_{n} k_{s} L/(k_{n} + k_{s}) = (1 + B_{min})/V_{n}$. Estimating $B_{min} = 10 \mu g \text{cm}^{-2} = 1 \times 10^{4} \text{molecules cm}^{-3}$ from Deen et al. (9) and using $k_{s} = 3 \times 10^{-12} \text{moleules cm}^{-1}$ yields $1 + B_{min} > 300$, which is surprisingly large.

We note that Deen et al. (9) stimulated the CD4⁺ lymphocytes in their assay with phytohemagglutinin and recent work by Gowda et al. (32) indicates that activation by mitogens increases the rate of CD4⁺ lymphocyte infection. Based on this, it is conceivable that activation increased both the probability of target cell infection, $i_{n}$, and the number of secondary virions, $V_{n}$. Experiments measuring the branching number of unstimulated and stimulated target cells are needed.

Since the branching number, $V_{n} = N V_{n} k_{s} L/(k_{n} + k_{s})$, is proportional to target cell concentration, we can extrapolate from the conditions of Deen et al. (9) ($L = 10^{6} \text{cells cm}^{-3}$ and $B_{min} > 10 \mu g \text{cm}^{-2}$) to the conditions in blood ($L = 10^{8} \text{cells cm}^{-3}$) and lymph node ($L = 10^{8} \text{cells cm}^{-3}$). Such extrapolation indicates a minimum therapeutic dose of $\approx 1000 \mu g \text{cm}^{-2}$ of sCD4 to treat established infections in vivo. Even more pessimistically, target cell infection from direct cell-to-cell contact (e.g., cell-mediated immunity or complex-restricted interactions) is probably less easily blocked than infection from the fluid medium. Experiments examining this situation are also required.

These results hold if the primary mechanism of action of sCD4 is simply to block the infectious process by steric hindrance. Siliciano et al. (33) and Lanzavecchia et al. (34) indicate that sCD4 may protect CD4⁺ lymphocytes from indirect or autoimmune effects of gp120. If this is the case, much lower concentrations of sCD4 may be of therapeutic use.

The branching process can also be used for estimating the immune response that an anti-gp120 vaccine must induce to protect against HIV infection. In this instance, $k_{s} = \text{approx} \text{constant between different cell-to-cell contact}$ (e.g., virus-mediated complex-restricted interactions) is probably less easily blocked than infection from the fluid medium. Experiments examining this situation are also required.

These results hold if the primary mechanism of action of sCD4 is simply to block the infectious process by steric hindrance. Siliciano et al. (33) and Lanzavecchia et al. (34) indicate that sCD4 may protect CD4⁺ lymphocytes from indirect or autoimmune effects of gp120. If this is the case, much lower concentrations of sCD4 may be of therapeutic use.

The branching process can also be used for estimating the immune response that an anti-gp120 vaccine must induce to protect against HIV infection. In this instance, $k_{s} = \text{approx} \text{constant between different cell-to-cell contact}$ (e.g., virus-mediated complex-restricted interactions) is probably less easily blocked than infection from the fluid medium. Experiments examining this situation are also required.

This is a pleasure to thank Peter Nara, Steven McDougal, Joseph Sodroski, and Raymond Sweet for stimulating discussions and data collection. This work was supported by the U.S. Department of Energy.