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Quantitative Model of Antibody- and Soluble CD4-Mediated Neutralization of Primary Isolates and T-Cell Line-Adapted Strains of Human Immunodeficiency Virus Type 1

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Primary isolates (PI) of human immunodeficiency virus type 1 (HIV-1) are considerably less sensitive than T-cell line-adapted strains to neutralization by soluble CD4 and by most cross-reactive monoclonal antibodies to the viral envelope (Env) glycoprotein, as well as by postinfection and postvaccination sera (J. P. Moore and D. D. Ho, AIDS 9 [suppl. A]:5117–5136, 1995). We developed a quantitative model to explain the neutralization resistance of PI. The factors incorporated into the model are the dissociation constants for the binding of the neutralizing agent to native Env oligomers, the number of outer Env molecules on the viral surface (which decreases by shedding), and the minimum number of Env molecules required for attachment and fusion. We conclude that modest differences in all these factors can, when combined, explain a relative neutralization resistance of PI versus T-cell line-adapted strains that sometimes amounts to several orders of magnitude. The hypothesis that neutralization of HIV is due to the reduction below a minimum number of the Env molecules on a virion available for attachment and fusion is at odds with single- and few-hit neutralization theories. Our analysis of these ideas favors the hypothesis that neutralization of HIV is instead a competitive blocking of interactions with cellular factors, including adsorption receptors.

The first strains of human immunodeficiency virus type 1 (HIV-1) used in neutralization experiments had been adapted in the laboratory to growth in immortalized T-cell lines (TCLA [T-cell line-adapted virus]) (reviewed in reference 34). These strains display variable, but often considerable, sensitivity to neutralization by sera from HIV-1-infected people, by monoclonal antibodies (MAbs) to several epitopes on the viral envelope glycoproteins (Env), and by soluble forms of the major receptor for the virus, CD4 (34). Primary HIV-1 isolates (PI), i.e., virus that has been recovered from patient material in cultures of peripheral blood mononuclear cells, with few passages, are generally insensitive to neutralization by both antibodies and soluble CD4 (sCD4) (3, 10, 33, 34, 36, 53). Although the role of neutralizing antibodies in the clearing of HIV-1 infections, in the abrogation of acute-infection viremia, and in curbing disease progression is uncertain (34), the neutralization insensitivity of PI has influenced the decision to interrupt clinical vaccine trials, which were monitored in part by testing for neutralizing antibodies (34). Other lentiviruses, such as simian immunodeficiency virus (SIV), feline immunodeficiency virus, and equine infectious anemia virus, have also been reported to lose their natural neutralization resistance upon serial passage in cell lines (5, 8, 34): a general explanation may be required.

Unlike several in vitro-selected neutralization-escape mutants of TCLA strains (34), the neutralization-resistant PI do not generally show a reduced affinity of their solubilized, monomeric outer Env proteins for the neutralizing agents (10, 33, 36). Furthermore, resistance of PI can be demonstrated with MAbs directed to multiple epitopes on the Env complex. Thus, the resistance can be global rather than restricted to local regions of Env (34). What could the mechanism of such general insensitivity be? The simplest explanation would be a difference in affinity of the neutralizing agents for native Env oligomers on the surface of the virion. Indeed, the oligomeric state of Env can affect the binding of neutralizing agents (6, 42, 49). Such an affinity difference could be due to quaternary-structural effects on the conformations of binding sites for the neutralizing agents or to the shielding of such sites by the quaternary interaction. In the former case, the poorly fitting binding sites may be in equilibrium with inducible better fits; in the latter case, the shielded state may be in equilibrium with an accessible one. But the two possibilities, misfit and shielding, would be thermodynamically indistinguishable: both would result in higher dissociation constants, K_d. However, there is empirical and theoretical support for a competitive mechanism of interference with Env binding to cellular CD4 by sCD4 (26) and by MAbs to the Env-binding site on CD4 (20, 21, 37). Yet changes in the affinity of Env for sCD4 would correlate with changes in its affinity for cellular CD4, although the latter may be an interaction of higher valency. The explanatory difficulty therefore emerges that when both the inhibitory and target interactions are weakened, no net insensitivity to neutralization should result. One possibility is that amino acid differences in Env epitopes for antibody binding may act selectively and leave the affinities of Env for cellular receptors intact, although the receptor-binding sites may overlap the epitopes. Thus, the oligomer shielding or misfit hypothesis may account for some of the observed neutralization insensitivity of PI. But can other mechanisms contribute? Can the relationship between various contributing parameters be expressed in one comprehensive formula?

The degree to which the surface glycoprotein of HIV-1, gp120 (SU), is associated with the transmembrane protein, gp41 (TM), varies between strains and between virion preparations of the same strain that have, for example, been kept at 37°C for different periods (24, 26, 32, 35, 36, 50). When shed-

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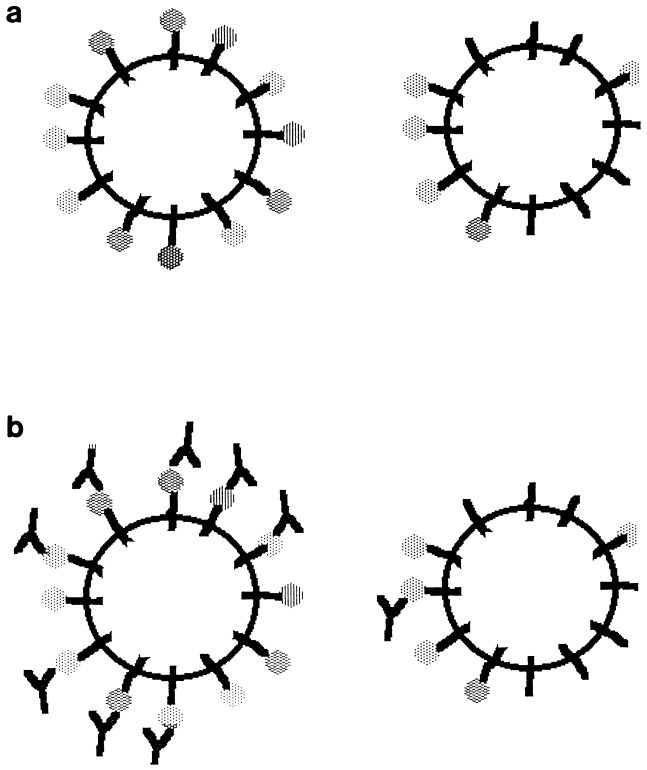


FIG. 1. (a) Two virions, the one to the left a PI virion, with a high density of spikes, or SU multimers, the one to the right a TCLA virion, with a low density. (b) As an example, the minimal spike number is here postulated to be 5, and so the maximal number of unoccupied spikes in the neutralized state is 4. The two virions as in panel a are now in the same neutralized state with minimal neutralizing concentrations. The relative occupancies, θ_{min}, however, are 0.67 for the left one and 0.2 for the right one. (θ_{min} is here crudely measured as occupied spikes over unoccupied plus occupied spikes; thus, relative occupancy for individual SU is not represented; see Materials and Methods). Potential effects of differential topographical distributions of spikes over the virion sphere are neglected here in the interest of simplicity.

ding of SU occurs, what effect does it have on neutralization? If the neutralizing agent is in vast molar excess over Env, even at its minimally effective concentration, then its absorption by one form of the antigen will only negligibly affect the degree of relative occupancy on another. In contrast, the number of spikes that remain on the virions may affect the minimum relative occupancy necessary for neutralization: the more such spikes, the higher the relative occupancy required. The prediction from this simple formulation (Fig. 1) agrees with the neutralization sensitivities of PI, which have a degree of SU-TM association approximately threefold higher than that of TCLA strains (32, 36, 39, 50). It also agrees with observed differences within the latter group, in which the extremely neutralization-sensitive strain SF-2 has a higher degree of spontaneous shedding than somewhat less sensitive strains like LAI and RF (29, 52). Furthermore, it agrees with observations on individual strains: the longer TCLA virions are incubated at 37°C, the more they shed SU and the more neutralization sensitive they become (24, 26).

MATERIALS AND METHODS

A mathematical formulation of the problem. What we seek to explain can be reformulated as a ratio of two concentrations: A_1, the minimal concentration of an agent needed to neutralize a certain infectious dose of a prototype TCLA strain, virus 1, and A_2, the corresponding concentration for the PI, virus 2. Thus designated, the empirically found ratio $A_2/A_1 >> 1$. A comprehensive model of neutralization sensitivity can now be mathematically expressed as the relation between the quantities outlined in the introduction, i.e., dissociation constants and total and minimal spike numbers.

The basic quantities and their relationships. We let the number of CD4-binding sites, SU monomers, on a virion = N. This number may vary from 0 to a maximal value, a multiple (depending on the oligomeric state of Env [21]) of 72 spikes per virion (15), or possibly greater for mutants with higher Env incorporation. It has been suggested that a critical or minimal number of Env molecules per virion must be unoccupied to give a high probability of infection (24–26). This critical number appears to be higher for infection of CEM-SS cells than of peripheral blood mononuclear cells by the TCLA clone HXB3 (24). We here define a distinct concept of absolute minimal SU numbers and apply that to the explanation of the relative neutralization insensitivity of PI. Thus, for a population of infectious virions, we hypothesize that N has a minimal value required for attachment and fusion. Let this minimal number of SU = n. We propose that this number is greater than the number of SU-TM molecules that directly form a fusion pore; other SU-TM molecules may be involved in attachment, and possibly only a subset of fusogenic conformational changes are productive. Furthermore, if spikes are attached to the underlying matrix protein, the majority of virions with patches containing enough spikes to form a fusion complex will have other spikes pointing in other directions.

Adaptation of HIV-2 and SIV_{mac} to growth in a T-cell line can also lead to increased neutralization sensitivity (34). Here the correlation between SU number and minimal neutralizing occupancy breaks down, for the sensitive SIV variant has the higher Env number per virion. The affinity of sCD4 and antibodies for the oligomeric Env rose concomitantly with T cell-line adaptation also for this variant (34). But if the affinity difference does not provide the whole explanation, the minimal spike number may contribute by being higher for the neutralization-sensitive variant.

The number of SU available for participation in a fusion complex can diminish by the shedding of SU from the virion, or by the binding to an SU of a neutralizing agent, which may or may not cause shedding (40). The relative occupancy, i.e., the proportion of SU monomers that have neutralizing molecules bound to them, θ, can be expressed as the ratio $\theta = (N - u)/N$, where u is the number of unoccupied, virion-bound SU. The maximal relative occupancy that will allow infection is then $\theta_{max} = (N - n)/N$.

We now let the maximal number of unoccupied SU compatible with the neutralized state = p. The minimal number of SU, n, required for infection is necessarily greater than p. Both numbers would be approximate averages for real virion populations. Not even in the improbable case of virions with identical patterns of spike distribution over the virion surface would n and p necessarily be integers; the relative contribution to attachment and fusion by a spike may depend on its distance from neighboring spikes, and so may the effects of the loss of a spike. The minimally neutralizing occupancy, θ_{min}, can now be expressed as

$$\theta_{min} = 1 - (p/N) \quad (1)$$

But according to the law of mass action (9, 19), the relative occupancy is a function of the dissociation constant, K_d, and the concentration of free neutralizing agent, A (all derivations in this work can be accessed via the internet at http://www.science.org/virology/) [hereafter referred to as http://www]): $\theta = (A/K_d)/(1 + (A/K_d))$. The A in this equation, the concentration of free neutralizing agent, is technically difficult to measure. However, when this agent is present in vast molar excess of the binding sites on the virus, the concentration of free agent can be approximated to the total concentration of agent, which is usually known. Incidentally, this analysis predicts a neutralization dependence on total antibody concentration up to extreme virus concentrations in accordance with the percentage law (2).

The minimal relative occupancy compatible with the neutralized state of the virus, θ_{min}, obtains at the minimal neutralizing concentration A_{min}. Some methods of determining this concentration are advantageous to modeling. The minimal concentration yielding complete neutralization of a predetermined infectious dose in an assay in which few infectious units are reproducibly detected diminishes the problem of heterogeneity of spike densities in virion populations: only virions in a narrow zone of the greatest resistance will require A_{min} for neutralization. But this virus may have a spike density higher than that of the average virus of the whole population. When instead the concentration of a neutralizing agent that yields a fixed percentage reduction of a large number of infectious units is measured, the model would apply to the virions in the borderline zone of neutralization. The model does not make predictions about concentrations of neutralizing agents required to yield certain decreases in antigen production, since such reductions may correspond to different numbers of infectious units for different strains. The minimal relative occupancy can be expressed

$$\theta_{min} = (A_{min}/K_d)/(1 + (A_{min}/K_d)) \quad (2)$$

By combining the two expressions for θ_{min} and solving for A_{min}, we obtain

$$A_{min} = K_d((N/p) - 1) \quad (3)$$

However, the theory is aimed at explaining a ratio of neutralization sensitivities, e.g., that observed between PI and TCLA strains. An expression is therefore

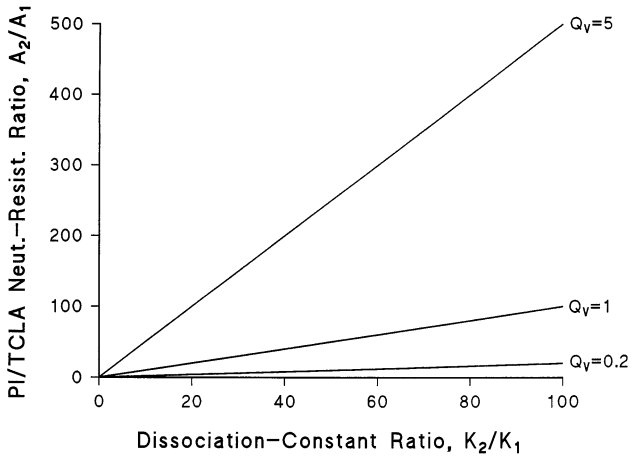


FIG. 2. Affinity of neutralizing agent for oligomeric Env influences neutralization in a linear fashion. The neutralization resistance ratio, A_2/A_1, expressed as a function of the ratio of dissociation constants, $f(K_2/K_1)$, is a proportionality: $A_2/A_1 = [((N_2/p_2) - 1)/((N_1/p_1) - 1)](K_2/K_1)$ or $A_2/A_1 = Q_v(K_2/K_1)$, with $Q_v = ((N_2/p_2) - 1)/((N_1/p_1) - 1)$, the virion factor, a constant the magnitude of which is determined by the relationship between, N, the total SU number and, p, the maximal number of unoccupied SU compatible with the neutralized state of PI and TCLA. Q_v is given three values, 0.2, 1, and 5.

sought for a ratio of two A_{min} values. The subscript 1 designates the variables and parameters pertaining to the TCLA, virus 1, and the subscript 2 designates those of the PI, virus 2. The relative neutralization resistance can then be expressed as a ratio:

$$A_2/A_1 = (K_2/K_1)((N_2/p_2) - 1)/((N_1/p_1) - 1) \quad (4)$$

Methods of affinity measurement. The biologically obtained affinity estimates (25) might seem the most relevant to the model. However, it can now be observed that these may agree better with the affinities for detergent-solubilized or recombinant gp120 than with those for virion-bound Env (33, 35, 43, 47). The current model may explain the value obtained by the biological method and how it relates to a true K_a: the biological association constant, K_a, for sCD4 binding to virion-bound gp120 is calculated as $((I_0/I) - 1)/[\text{sCD4}]$ (25), where I_0 is the control infectivity in the absence of sCD4, I is the infectivity in the presence of sCD4, and [sCD4] is the sCD4 concentration. Thus, the formula $K_a = ((I_0/I) - 1)/[\text{sCD4}]$ gives the slope of the curve describing I_0/I as a function of [sCD4]. What is required for the derivation of the formula, in the current symbolism, is that $(I_0/I) = 1/(1 - \theta)$, i.e., that the relative infectivity, (I/I_0), equals the relative nonoccupancy, $1 - \theta$. However, the kinetic model of independent and equivalent gp120 molecules (27) is at odds with hypothesizing threshold numbers of SU or else does not entail $I/I_0 = 1 - \theta$. Invoking the relative occupancy-based model, we argue that in a heterogeneous population of virions, there exist some with a spectrum of SU numbers close to the minimal one and that these virions are gradually knocked out as the concentration of sCD4 rises. It has indeed been observed that the slope $((I_0/I) - 1)/[\text{sCD4}]$ is affected by quantities other than affinity, e.g., gp120 retention (see Fig. 8 in reference 24). The biological affinity measurement leads to an estimation of the critical SU number to 50 ± 25 per virion (24). The physicochemical value of $K_a \approx 10^{-8}$ M at 37°C (35) would give a critical SU number of 90 ± 45. We suggest that physicochemically determined affinities of antibodies and sCD4 for virion-bound oligomeric Env ought to be used in order to avoid circularity and internal contradictions in the model. We acknowledge, however, the technical difficulties in obtaining such values accurately (43).

Effects of affinity differences on the neutralization sensitivity ratio. In the following, all functional transformations were performed and curves were plotted by the use of the program FigP (Biosoft, Cambridge, England), with minimal unit steps of the x values where applicable.

In the current model, effects of the affinities of neutralizing agents for oligomeric virion-bound SU would be seen in isolation with pairs of viruses for which the factor $((N_2/p_2) - 1)/((N_1/p_1) - 1)$ in equation 4 is kept constant. Call this constant Q_v, the virion constant. As seen from equation 4 and illustrated in Fig. 2, the neutralization resistance ratio as a function of the dissociation constant ratio, $A_2/A_1 = f(K_2/K_1)$, is a proportionality. That Q_v has the power to magnify or shrink the affinity effect, but not to obliterate it, is illustrated by curves for a sample of different Q_v values in Fig. 2. Q_v is equal to unity for a virus pair with identical total numbers of SU and identical maximal unoccupied numbers of SU at neutralization. In practice, this situation may seldom obtain since, for example, a mutation that changes the affinity may also affect the total and minimal SU numbers. It is less trivial that pure affinity effects would also obtain when the pairs of compared viruses have the same "relations" between their total SU numbers and maximal unoccupied SU numbers at neutralization. In both situations, the term Q_v equals unity, although the physical states of the virions could differ greatly.

In conclusion, this analysis shows that affinity effects on neutralization resistance are proportional: for virions with identical minimal and actual SU numbers, an affinity ratio of 100 is required to explain a relative neutralization resistance of 100.

Dissecting the effects of the virion factor. It is apparent that the ratio A_2/A_1 is a linear function of some of the quantities in the virion factor $Q_v = ((N_2/p_2) - 1)/((N_1/p_1) - 1)$ made variables but a nonlinear function of others. $f(N_2) = (K_2/K_1)((p_1/p_2)/(N_1 - p_1))N_2 - (K_2/K_1)(p_1/(N_1 - p_1))$ is linear. The slope of the curve is determined by the relationship between the dissociation constants, the maximal unoccupied SU number of PI, virus 2, and both the total and maximal unoccupied SU numbers of TCLA, virus 1, viz., the factor $(K_2/K_1)(p_1/p_2)/(N_1 - p_1)$. The plot of $A_2/A_1 = f(N_2)$ is shown in Fig. 3a. The plot in Fig. 3a means that, for example, if the TCLA had 60 SU per virion, the maximal unoccupied SU number being 40 for both TCLA and PI, all other things being equal, then the PI would have to have 240 SU per virion in order to yield a 10-fold relative neutralization resistance.

In contrast, the neutralization resistance ratio as a function of the total SU number of the TCLA, $f(N_1) = (K_2/K_1)(p_1/p_2)(N_2 - p_2)(1/(N_1 - p_1))$, is nonlinear, a rectangular hyperbola. As illustrated in Fig. 3b, the difference in neutralization resistance increases drastically when the total number of SU on the first virus approaches its maximal unoccupied SU number; $A_2/A_1 \rightarrow \infty$ as $N_1 \rightarrow p_1$. The plot in Fig. 3b means, for instance, that when the number of SU on the TCLA is 2 SU above the maximal unoccupied SU number, and the maximal number of unoccupied SU, p, is 40 for both viruses, then the relative neutralization resistance of a PI with 200 SU rockets to 80-fold.

Since a difference in total SU numbers influences A_2/A_1, we let $N_2 = N_1 + \Delta N$ and express A_2/A_1 as $f(\Delta N) = (K_2/K_1)((p_1/p_2)/(N_1 - p_1))\Delta N + (K_2/K_1)(p_1/p_2)(N_1 - p_2)/(N_1 - p_1)$. Thus, again, a linearity is obtained, and one with the same slope as $f(N_2)$, as illustrated in Fig. 3c. The plot in Fig. 3c means that to explain a 10-fold neutralization resistance, when the maximal number of unoccupied SU, p, is 40 for both PI and TCLA, and when the TCLA has 60 SU per virion, the PI would need to have 180 SU more, i.e., a total of 240 SU, or 4-fold more than the TCLA. Experimental measurements of N_2/N_1 are typically around 3 (32, 36, 39, 50).

What happens to the neutralization resistance ratio when N_1 and N_2 are kept constant and p_1 and p_2 are varied one at a time? $f(p_2) = (K_2/K_1)(p_1N_2/(N_1 - p_1))(1/p_2) - (K_2/K_1)(p_1/(N_1 - p_1))$ is hyperbolic (Fig. 4a). This is significant, as it is the only hyperbolic function in which the variable is derived from the numerator, i.e., the expression pertaining to A_2. The hyperbolic relationship therefore here expresses an intrinsic property of the absolute neutralization resistance of virus 2, the PI in the example, rather than the relative resistance affected by the properties of the TCLA strain. The middle curve in Fig. 4a, for example, means that if the TCLA has a maximal unoccupied SU number of 40 and a total SU number of 60, and the PI has a total of 200 SU per virion, the relative neutralization resistance reaches about 10 as the maximal unoccupied SU number of the PI sinks to 33.

As shown in Fig. 4b, the neutralization resistance ratio is relatively unaffected by changes in the maximal number of unoccupied SU on neutralized TCLA virions, p_1, until p_1 enters a zone close to N_1, where a dramatic increase occurs: $f(p_1) = (K_2/K_1)((N_2 - p_2)/p_2)(1/((N_1/p_1) - 1))$. The plot in Fig. 4b thus means that when the PI has a total SU number of 200 and a maximal unoccupied one of 40, and the TCLA has a total SU number of 60, then if the TCLA maximal unoccupied SU number were set to, for example, 58, the relative neutralization resistance of the PI would be 116-fold.

If A_2/A_1 is expressed as a function of Δp by arbitrarily letting $p_1 = p_2 + \Delta p$, where $\Delta p \geq 0$, we obtain $f(\Delta p) = (K_2/K_1)((N_2 - p_2)/p_2)(1/(N_1/(p_2 + \Delta p) - 1))$. Thus, again, the function is hyperbolic: as $p_2 + \Delta p \rightarrow N_1$, $(A_2/A_1) \rightarrow \infty$, which is plotted in Fig. 4c. A_2/A_1 is greater the higher the parameter p_2 is set, the converse of Fig. 3b. This is because we have made p_1 automatically increase with p_2, and thus approaching N_1. As an example, the plot in Fig. 4c means that if PI has a maximal unoccupied SU number of 50 and a total SU number of 200, while the TCLA has a total SU number of 60, then as the maximal unoccupied SU number of the TCLA is imagined to be 8 SU higher than that for the PI, the relative neutralization resistance of the PI would be around 90-fold.

All of the effects of variations in K, N, or p on the relative PI/TCLA neutralization resistance would be dampened by the neutralizing agent binding, or acting, with positive cooperativity and enhanced by negative cooperativity (9).

The molecularity of neutralization. Except for the case of virions with on average one or a few SU, or spikes, above the minimal number required for infection, the present model is incompatible with single- or few-hit hypotheses of neutralization. Dulbecco et al. (13) described the neutralization reaction of antibodies with both the enveloped western equine encephalitis virus and the naked poliomyelitis virus as being of first-order kinetics (13). They interpreted this as meaning that the neutralization was a single-hit reaction. Since then, single- and few-hit neutralization has been hypothesized for various viruses, including the enveloped influenza virus and HIV-1 (30, 51). In a few instances, stoichiometric measurements have been made by use of radiolabeled antibodies.

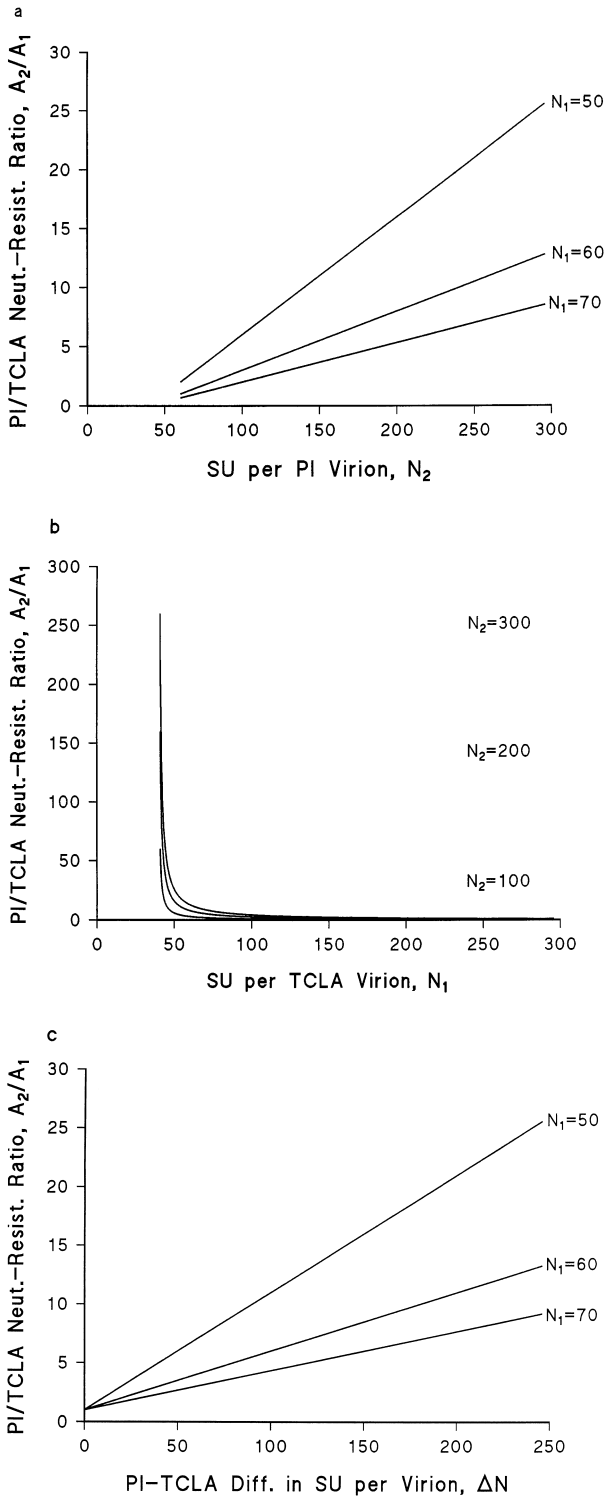


FIG. 3. How neutralization sensitivity is expected to vary with the total number of SU per virion. The effects of changes in the total number of SU per virion, N, on the neutralization resistance ratio, A_2/A_1, is illustrated for the N of each virus and for the difference between them. It should here be borne in mind that each spike is an oligomer of SU-TM. (a) A linear relationship is found between N_2, the total number of SU on the less sensitive virions, PI, and the neutralization resistance ratio, $A_2/A_1 = f(N_2)$. $A_2/A_1 = (N_2 - 40)/(N_1 - 40)$ when $K_2/K_1 = 1$, $p_1/p_2 = 1$, and $p_1 = 40$. N_1, the total number of SU on the TCLA virions, is given three values, 50, 60, and 70. (b) The neutralization resistance ratio is found to have a rectangular hyperbolic relationship to the total number of SU on the more sensitive, TCLA virus, $A_2/A_1 = f(N_1)$: $A_2/A_1 = (N_2 - 40)\ (1/(N_1 - 40))$ when the parameter values are $K_2/K_1 = 1$, $p_1/p_2 = 1$, and $p_1 = 40$. $N_2 = 300$ (upper curve); $N_2 = 200$ (middle curve); $N_2 = 100$ (lower curve). N_1 is varied in the interval $41 \leq N_1 \leq 296$. The catastrophic changes in sensitivity occur when N_1, the total number of SU on the more sensitive virus, gets close to its maximal number of unoccupied SU compatible with neutralization, p_1, which we have set to 40 in this simulation. (c) The dependence of the neutralization resistance ratio on the difference in total number of SU per virion between the two viruses is linear, $A_2/A_1 = f(\Delta N)$. $K_2/K_1 = 1$, $p_1/p_2 = 1$, and $p_1 = 40$ give $A_2/A_1 = [1/(N_1 - 40)]\Delta N + 1$. N_1 is given three values, 50, 60, and 70.

Thus, the minimal binding of 4 antibodies to poliovirus (17) and of 70 antibodies to influenza virus (51) has been shown to be required for neutralization reactions that proceed with first-order kinetics. This has been said to constitute a paradox (12). As a resolution of the paradox, it has been hypothesized that only a small fraction of the epitopes that bind neutralizing antibodies are neutralization relevant (17, 51).

A reaction of first order in antibody concentration can result from a multihit reaction (4). Bimolecularity of a neutralization reaction, i.e., one antibody molecule neutralizing one virion, implies first-order kinetics in either concentration, but the reverse is not true (4). Evidence for the molecularity of neutralization reactions can be obtained from stoichiometric measurements, which have been made, for example, by the use of radiolabeled antibodies (17, 51). Such evidence can be interpreted on the basis of the statistically plausible Poisson distribution of levels of antibody binding to virions (14). We let $P(h)$ be the probability that a virion suspended in an antibody solution has h immunoglobulin G (IgG) molecules bound to it (not differentiating between one paratope, the other paratope, or both paratopes being bound). If h_{min} is the minimal number of bound antibodies required to neutralize a virion, I is the number of infectious units remaining after neutralization, and I_0 is the original number of infectious units, controlled for "spontaneous" decline during the neutralization reaction, then I/I_0 is the sum of the probabilities of all states of a lower number of antibodies bound than required for neutralization, including none bound: $I/I_0 = P(0) + P(1) + P(2) + P(3) + \ldots + P(h_{min} - 1)$.

Generally, $P(h) = (e^{-m}m^h)/h!$ (14), where m is the average number of antibodies bound per virion: $I/I_0 = e^{-m} + e^{-m}\,m + (e^{-m}\,m^2)/2! + (e^{-m}\,m^3)/3! + \ldots + (e^{-m}\,m^{h_{min}-1})/(h_{min} - 1)!$ When m is measured directly, or when the concentration of free antibodies is varied and m is calculated, the natural logarithm of I/I_0 can be plotted as a function of m, $f(m) = \ln(I/I_0)$. As illustrated in Fig. 5a, $h_{min} = 1$ gives a straight line, since then $I/I_0 = P(0)$, and thus $f(m) = -m$. By an analysis based on a linear best fit and reading of the average antibody number for a relative infectivity $1/e$, the minimal number of antibody molecules for poliovirus was previously determined to be 4 (17). We suggest that such an analysis is justified only in testing a single-hit curve. It is merely a coincidence that this method gives a value close to what an orthodox Poisson-based analysis would give. When a virus requires a greater minimal number of antibodies for neutralization, the corresponding deviation would be larger. This is pertinent to future stoichiometric analyses of HIV and SIV neutralization.

Measurements of m are, however, often not available, but the total antibody concentration, A_{tot}, i.e., bound plus unbound, or the serum dilution, has to be used instead. Then, if the concentration of epitopes on the virions and the functional affinity are known, m can be calculated, and $\ln(I/I_0)$ can be expressed as a function of A_{tot}. Assuming, as an example, 80 SU molecules per HIV-1 virion and, for simplicity, that this is equivalent to 40 antibody-binding sites (with the stipulated values of the dissociation constant for antibody binding and the concentration of virion SU both set to 0.5 [nM]; see http://www), one gets

$$I/I_0 = e^{-40\,(A_{tot} + 1 - [A_{tot}^2 + 1^{1/2}} + e^{-40(A_{tot} + 1 - [A_{tot}^2 + 1]^{1/2})}\,(40\,(A_{tot} + 1 - [A_{tot}^2 + 1]^{1/2}))/1!$$
$$+ (e^{-40\,(A_{tot} + 1 - [A_{tot}^2 + 1]^{1/2})}\,(40\,(A_{tot} + 1 - [A_{tot}^2 + 1]^{1/2}))^2)/2!$$
$$+ (e^{-40\,(A_{tot} + 1 - [A_{tot}^2 + 1]^{1/2})}\,(40\,(A_{tot} + 1 - [A_{tot}^2 + 1]^{1/2}))^3)/3! + \ldots$$
$$+ (e^{-40\,(A_{tot} + 1 - [A_{tot}^2 + 1]^{1/2})}\,(40\,(A_{tot} + 1 - [A_{tot}^2 + 1]^{1/2})^{(h_{min} - 1)})/(h_{min} - 1)! \quad (5)$$

Now $\ln(I/I_0)$ can be expressed as a function of A_{tot}, $f(A_{tot})$. As illustrated for A_{tot} ranging from 0 to 5 mM in Fig. 5b, and for a more narrow range of antibody concentration in Fig. 5c (A_{tot} range, 0 to 0.5 mM), this relationship is not linear even for $h_{min} = 1$ but is more approximately so in the low A_{tot} range for $h_{min} = 1$ than for higher h_{min} values.

An occupancy analysis of few-hit hypotheses shows (Fig. 5b) that relative infectivities will drop tens of natural logarithms after virus incubations with antibody concentrations in a zone between K_d and 10 K_d (and this in spite of the high virion-bound SU concentration of 0.5 nM). For PI or other HIV-1 isolates with more than 80 SU per virion, the predicted drops would be even more drastic. Thus, the relative and absolute neutralizing concentrations for PI and TCLA that few-hit hypotheses imply are at odds with empirical data (20, 22, 30, 43).

We conclude from this analysis that there are no empirical or theoretical impediments to a multihit neutralization model that invokes blocking of Env

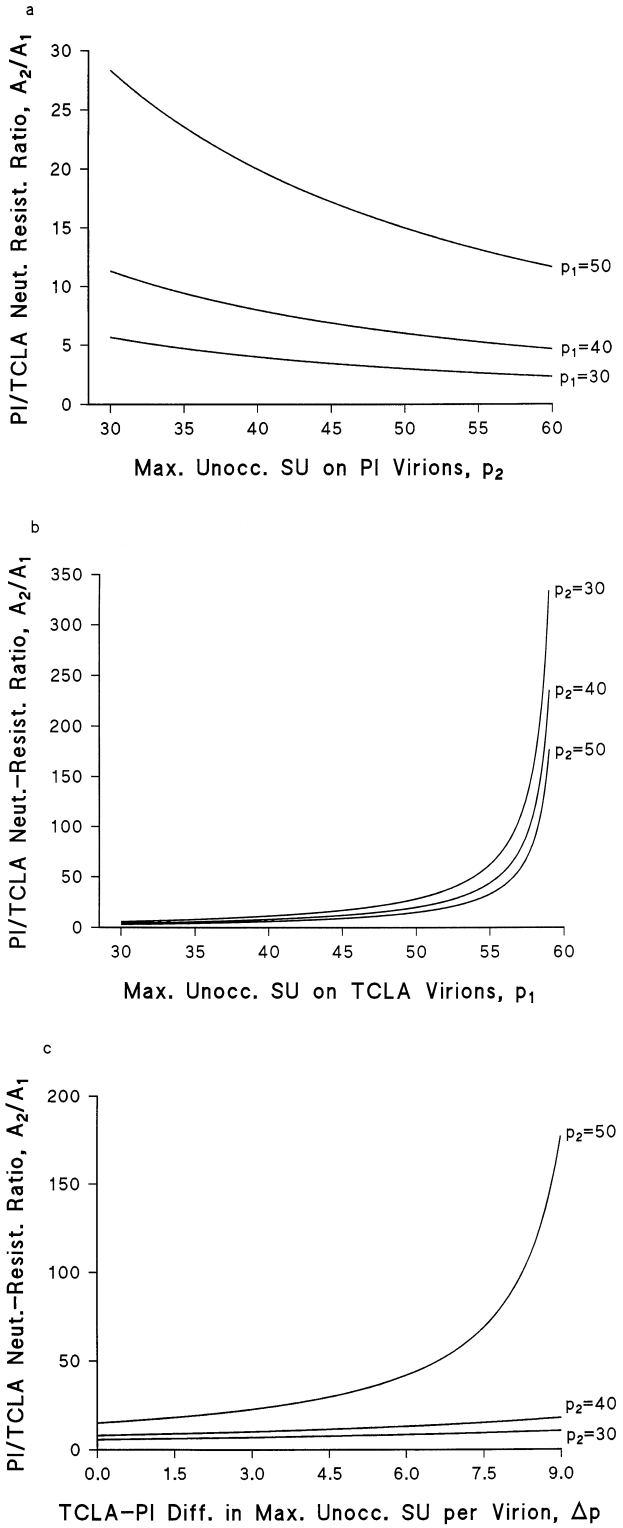


FIG. 4. (a) The maximal number of unoccupied SU compatible with the neutralized state is varied for PI, the less sensitive virus. The relationship of the neutralization resistance ratio to this variable is illustrated, $A_2/A_1 = f(p_2)$. The parameter values $K_2/K_1 = 1$, $N_1 = 60$, and $N_2 = 200$ give $A_2/A_1 = [(200p_1/(60 - p_1))(1/p_2)] - (p_1/(60 - p_1))$. p_1 is given three values, 30, 40, and 50. The lower the variable, the higher the neutralization resistance ratio. This means that the fewer SU that PI, virus 2, needs for infectivity, the less affected it would be by a certain concentration of neutralizing agent. It can be seen that although the relationship to p_2 is hyperbolic, no drastic effects occur unless the corresponding quantity for the more sensitive TCLA virus, p_1, approaches the total number of SU per virion for that virus. (b) The effects of changes in the maximal unoccupied SU number, p_1, of the more sensitive virus are opposite to, and more catastrophic than, the corresponding changes for the less sensitive virus. $A_2/A_1 = f(p_1)$ can be written in the form $A_2/A_1 = (K_2/K_1)((N_2 - p_2)/p_2)(1/((N_1/p_1) - 1))$. With the parameter values analogous to those in panel a, $K_2/K_1 = 1$, $N_1 = 60$, and $N_2 = 200$, we get the expression $A_2/A_1 = ((200 - p_2)/p_2)(1/((60/p_1) - 1))$. p_2 is given three values, 30, 40, and 50. (c) If A_2/A_1 is expressed as a function of Δp, $f(\Delta p)$, by arbitrarily letting $p_1 = p_2 + \Delta p$, and the parameter values are $K_2/K_1 = 1$, $N_1 = 60$, $N_2 = 200$, we obtain $A_2/A_1 = ((200 - p_2)/p_2)(1/(60/(p_2 + \Delta p) - 1))$. p_2 is given three values, 30, 40, and 50. Δp is varied from 0 to 9. As $p_2 + \Delta p \rightarrow N_1$, $(A_2/A_1) \rightarrow \infty$: shrinking the number of SU that the TCLA has in excess of its maximal unoccupied number yields drastic changes in neutralization sensitivity. With $p_2 = 30$ or 40, this excess does not get small enough in the range of the variable plotted for the drastic changes to occur.

protein function. This opens the possibility of a competitive mechanism of neutralization (26, 48). One of the possible steps in viral entry that could be competitively blocked is adsorption.

Adsorption limitation and infectivity per virus particle. The infectious fractions of both TCLA and PI have been found to adsorb so inefficiently to target cells that the specific loss of infectivity is not detectable even after 2 h. To explain this low level of adsorption, inefficient adsorption steps preceding CD4 binding were postulated (18). What degree of virus adsorption should be expected if the SU interaction with CD4 were the sole means of virus attachment to cells? We attempted an abstract simulation of HIV adsorption, in which virion-bound SU and cellular CD4 are postulated to behave like molecules in solution, albeit with a 10-fold-higher functional affinity, as a correction for multivalency (see http://www). We found that the concentrations of the two proteins in ordinary infectivity assays are so low in relation to the K_d that the fraction of virions adsorbed could indeed plausibly be undetectable. The need for postulating other interactions that interfere with SU-CD4 binding (18) may thereby be diminished.

In theory, the binding of Env to an additional receptor might decrease the affinity of SU for CD4. But such an effect would not significantly affect the binding to CD4 unless the additional factor had a binding capacity, i.e., abundance and affinity, comparable to that of CD4. Thus, again, no net decrease in adsorption is predicted on such grounds. If a factor is needed to explain a lower than predicted adsorption, then a negative factor, such as a barrier due to electrostatic repulsion or an oligomeric Env structure with a reduced functional affinity for CD4, might be invoked. Accessory receptors would have to be taken account of in any rigorous model of HIV-1 attachment and fusion. But their additional adsorptive effects, even if they act with extremely low affinities, increase the problem of explaining inefficient HIV-1 adsorption, as can be shown by analysis of linked equilibria (http://www).

Competition between neutralizing agents and cellular receptors for binding to SU. The intrinsic affinity of oligomeric PI Env for CD4 may be lower than that of TCLA Env, or the on-rate constant may be lower (36), giving lower preequilibrium levels of binding. It is plausible that a higher spike density could compensate for this by raising the functional affinity of the Env-CD4 binding. If so, it would also explain why the lymphocytotropic PI can take advantage of increased CD4 concentrations at the cell surface, an ability that is lost by selection for growth in T-cell lines with lower cell surface levels of CD4 (18). These counteracting effects could therefore result in little net difference between PI and TCLA in their abilities to attach to cells. But how should the consequences for neutralization be analyzed? We incorporated competition between a neutralizing agent and cellular receptors into equation 4 by modeling the limiting case of indeterminate infectivity when $|p - n|_{\lim} \rightarrow 0$ (equations available via http://www). Such competition between neutralizing agent and cellular receptors would convert the linear dependence on ΔN of Fig. 3c to a hyperbolic one. The difference in total number of SU would give progressively higher ratios of neutralizing concentrations. We cannot, on the basis of available data, adjudicate between the description in Fig. 3c and one that takes competition into account: they may be seen as the ends of a spectrum of hypotheses. The competition model necessitates further assumptions that are hard to justify experimentally. Therefore, we merely point out that the introduction of a competitive element may render the plot in Fig. 3c a better approximation to the truth. The competition analysis implies that if the argument can be extended to bivalently binding antibodies, PI will show greater relative resistance to neutralizing antibodies the greater the competitive component in their mechanism of action is. Conversely, the greater the valency of the neutralizing agent is, the more the relative neutralization resistance of PI versus TCLA would be reduced. This conclusion is corroborated by the lower A_2/A_1 for a tetravalent CD4-IgG construct than for monovalent sCD4 (1).

Schematic of the modeling steps. (i) The relative PI/TCLA neutralization resistance was formulated as a ratio of minimal inhibitory concentrations of a neutralizing agent. (ii) The explanation was derived from the law of mass action and the concepts of total SU number per virion, minimal SU number per virion required for infectivity, and maximal number of unoccupied SU compatible with the neutralized state. (iii) The resulting model yielded a linear dependence of the neutralization resistance ratio on affinity, on the total SU number of PI, and on

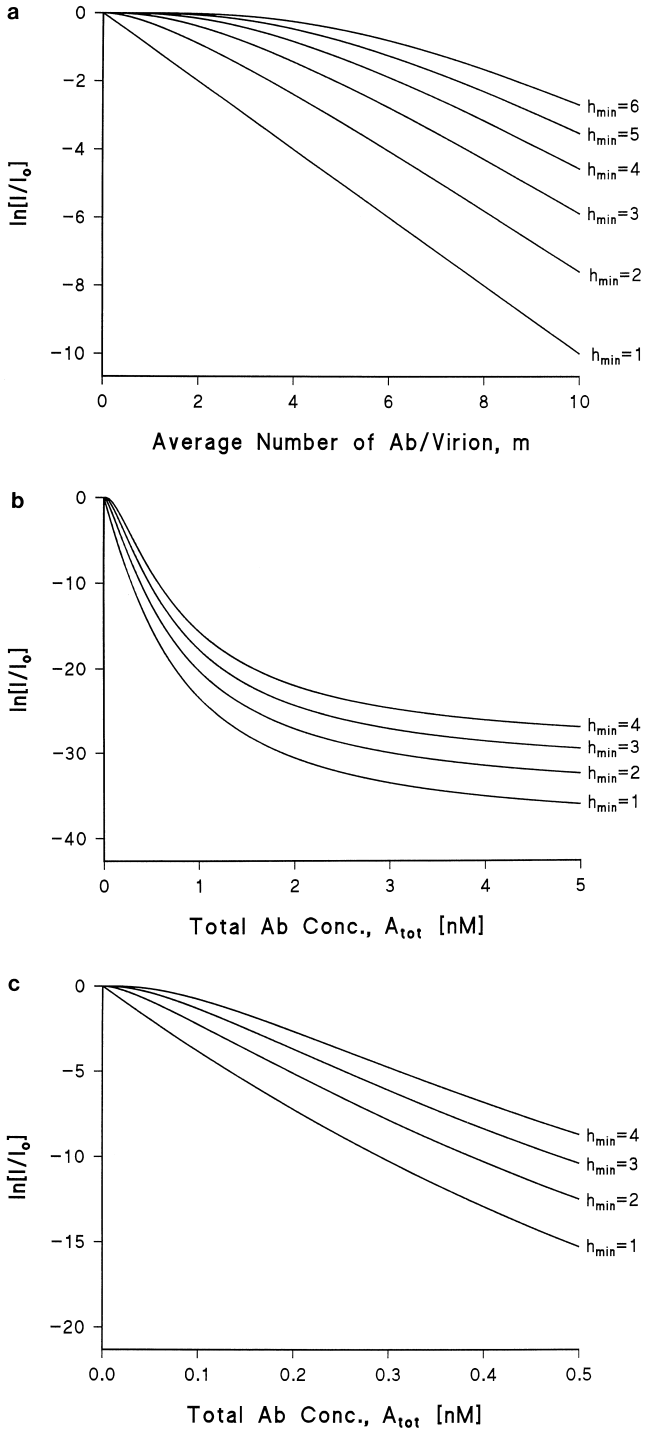


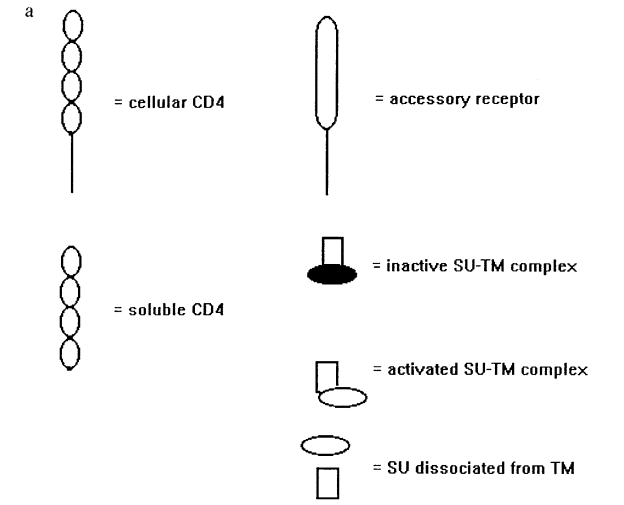
FIG. 5. Few-hit theories of neutralization investigated by Poisson-based analysis. (a) The relationship between the natural logarithm of the relative residual infectivity, $\ln(I/I_0)$, is plotted as a function of the average number of antibody molecules bound per virion, m. Theoretically, the probability $P(h)$ of having h antibodies bound on a virion, with the average m, is $P(h)=(e^{-m}m^h)/h!$, according to the Poisson distribution. If we stipulate that h_{min} is the minimal number of antibodies bound to a virion required for neutralization, then the natural logarithm of the residual infectivity after the neutralization reaction is $\ln(I/I_0) = \ln[e^{-m} + e^{-m}\,m + (e^{-m}\,m^2)/2 + (e^{-m}\,m^3)/6 + \ldots + (e^{-m}\,m^{h_{min}-1})/(h_{min}-1)!]$. $\ln(I/I_0) = f(m)$ is plotted for h_{min} = 1, 2, 3, 4, and 5. Thus, h_{min} = 1 gives a linear relationship because then $\ln(I/I_0) = -m$. (b to c) Poisson analysis applied to HIV-1 neutralization. The natural logarithm of the relative residual infectivity is plotted as a function of the total concentration of neutralizing antibody, $f(A_{tot}) = \ln(I/I_0)$. If the total number of antibody-binding sites is 40, then $m = 40\theta_v$. $\theta_v = ((((A_{tot} + K)/V_{tot}) + 1)/2) - [((((A_{tot} + K)/V_{tot}) + 1)/2)^2 - A_{tot}/V_{tot}]^{1/2}$ was simplified to $\theta_v = A_{tot} + 1 - [A_{tot}^2 + 1]^{1/2}$ by letting $K = 0.5$ [nM] and $V_{tot} = 0.5$ [nM]. Thus, $m = 40(A_{tot} + 1 - [A_{tot}^2 + 1]^{1/2})$, which substituted into the expression for the natural logarithm of the relative residual infectivity in panel a gives $f(A_{tot}) = \ln[e^{-40(A_{tot} + 1 - [A_{tot}^2 + 1]^{1/2})} + e^{-40(A_{tot} + 1 - [A_{tot}^2 + 1]^{1/2})}\,(40(A_{tot} + 1 - [A_{tot}^2 + 1]^{1/2})) + (e^{-40(A_{tot} + 1 - [A_{tot}^2 + 1]^{1/2})}\,(40(A_{tot} + 1 - [A_{tot}^2 + 1]^{1/2}))^2)/2 + (e^{-40(A_{tot} + 1 - [A_{tot}^2 + 1]^{1/2})}\,(40(A_{tot} + 1 - [A_{tot}^2 + 1]^{1/2}))^3)/6 + \ldots + (e^{-40(A_{tot} + 1 - [A_{tot}^2 + 1]^{1/2})}\,(40(A_{tot} + 1 - [A_{tot}^2 + 1]^{1/2}))^{h_{min}-1})/(h_{min}-1)!]$. (b) $f(A_{tot})$ is plotted with A_{tot} in the range 0 to 5 mM for h_{min} = 1, 2, 3, and 4. Unlike in panel 1, there is no linear relationship for any h_{min}. (c) With A_{tot} in the range 0 to 0.5 mM, $f(A_{tot})$ is only approximately linear for h_{min} = 1 and exhibits a shoulder for h_{min} = 2, 3, or 4.

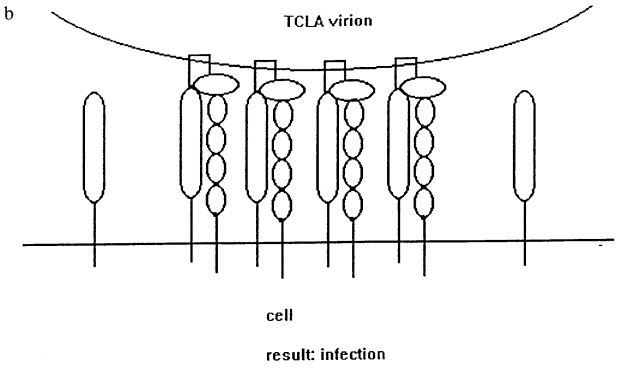
the difference in total SU number between PI and TCLA. Hyperbolic increases in the ratio were seen with increased maximal unoccupied SU number on the TCLA. Hyperbolic decreases were seen with increases in the total SU number on the TCLA and the maximal unoccupied SU number on the PI virions. (iv) Except in extreme cases, the model would be incompatible with single- and few-hit hypotheses of neutralization, which we investigated by finding an expression for the logarithm of surviving infectious fractions for HIV-1 according to the Poisson-distributed binding of few antibodies under realistic assumptions of SU numbers. The thus predicted degrees of neutralization disagree with available data, which may favor the alternative theory, viz., neutralization as sufficient relative occupancy for bringing the number of functional Env molecules below a minimum, a hypothesis which could allow for competition with cell-factor interactions, e.g., those involved in adsorption.

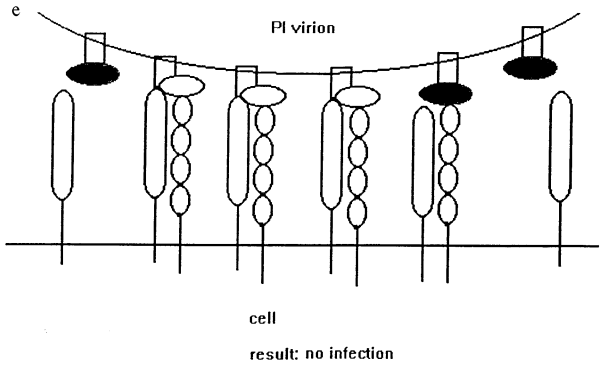
RESULTS AND DISCUSSION

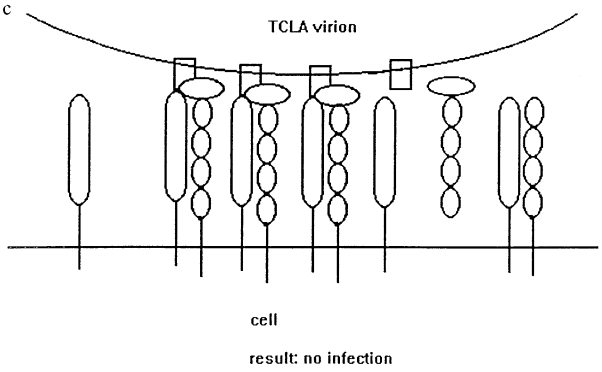
We have here derived a quantitative relationship between neutralization sensitivities and Env-ligand affinities, the total number of SU on virions, and the minimal number of functional SU-TM molecules required for infection. We deem all of these factors important in creating the up to 1,000-fold PI/TCLA neutralization resistance ratio (3, 7, 10, 50, 53). The premises are thermodynamic and biological. They do not explicitly include kinetic factors, although these of course determine the equilibrium constants. Nevertheless, we predict that kinetic differences in the binding of such agents to virions will influence neutralization in proportion to their effect on the relative occupancy that is achieved when virion meets cell. Thus, the shorter the preincubation of virus with a neutralizing agent before mixing with cells, the more profoundly would the on-rate constant of the binding of the neutralizing agent influence neutralization. Indeed, a recent study suggests that some neutralizing MAbs have particularly low on-rate constants for PI and that the on-rate constant may correlate with potency of neutralization (43). Our model is in principle amenable to letting the on- and off-rate constants determine the preequilibrium occupancies at various stages. The simplification involved in assuming near equilibrium does not affect the gist of the model.

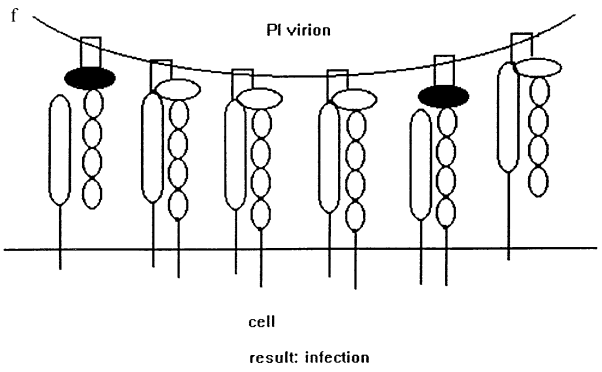
For HIV-1 (30) and other viruses (reviewed in reference 11), an initial shoulder on the curve describing the logarithm of relative residual infectivity as a function of time has been interpreted as evidence for multihit neutralization. Conversely, the lack of such a shoulder has been taken as evidence for a single-hit mechanism of neutralization by the antibody, e.g., one against the CD4-binding site on HIV-1 SU (30). We suggest that since the spike density of a population of virions is heterogeneous (15, 24), there may be a few virions with an SU number just above the minimum. The neutralization of these may begin early, since it would require only one hit. Although the model implies that the few virions with minimal SU numbers will effectively be neutralized by a single-hit mechanism, the conjecture of this limiting case is very different from the hypothesis of a single-hit mechanism that invokes a global effect on the virion of the binding of one antibody (12).

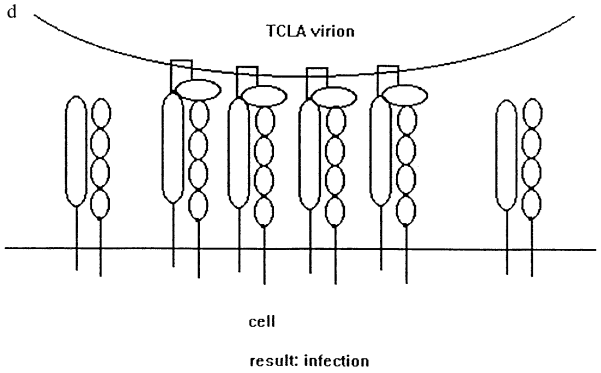












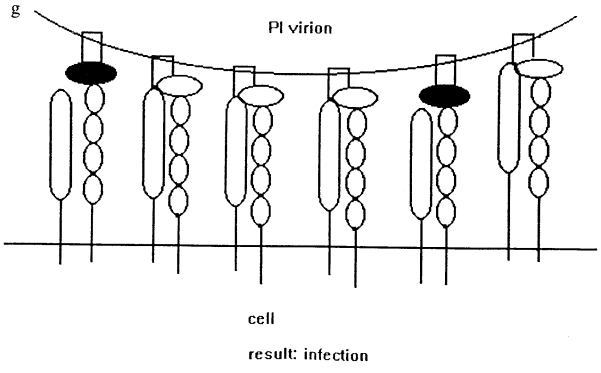


FIG. 6. Iconic modeling of PI-TCLA differences in CD4-mediated activation of Env fusogenicity, dependence on cellular CD4 density, and sCD4 enhancement of infection. (a) The quaternary structure of Env has here been disregarded for clarity but could be pictured as involved in the projected differences in CD4 induction of the fusogenic potential of Env; e.g., a greater relative occupancy of CD4 on the Env oligomers may be required for PI fusion activation than in the case of TCLA. For simplicity, the minimum number of SU-TM involved in a fusion complex is postulated to be the same for PI and TCLA. For pictorial clarity, this number is set to a possibly unrealistically low number, four. With varying specific fusogenicities, identical SU-TM numbers in fusion complexes mean that different minimum SU numbers per virion, n, would be required to create active fusion complexes. The situation is modeled from attachment onward; i.e., also in the interest of simplicity, the propensity to attach, which the PI and TCLA virions would have at the lowest cellular CD4 density, is not modeled differently for PI and TCLA; events that may arise with different probabilities are instead modeled in parallel. The hypothetical accessory receptor for HIV-1 is pictured to be in excess of the lowest CD4 levels but to lack significant affinity for Env in isolation. (b to d) TCLA. (b) A TCLA virion is attached to a susceptible cell via four SU-TM complexes, all of which are activated. This is postulated to be the minimum size of the fusion complex, and infection results. (c) sCD4 is present at a concentration yielding a relative occupancy $\theta = 0.25$ on the SU. The binding of sCD4 to an SU has induced its dissociation. As a consequence, only three cellular CD4-SU-TM complexes are formed, i.e., one short of a fusion complex. Therefore, no infection results. (d) The cell surface concentration of

CD4 is 50% higher than in panels b and c. Because of the low SU density on the TCLA virion, no additional contacts arise. The result is infection, with the same probability as in panel b. (e to g) PI. (e) The spike density on this PI virion is only 50% higher than that on the TCLA virion. Four contacts between SU and CD4 are established. However, because of the higher threshold of PI SU-TM to fusogenic triggering, only three of the four liganded SU become activated, i.e., one short of a fusion complex. No infection results. (f) sCD4 is present at a concentration yielding a relative occupancy $\theta = 0.33$ on the virion-bound SU. One of two SU-TM is activated by sCD4. (The other one could have been unchanged [shown] or, alternatively, abortively changed, e.g., by sCD4-induced shedding [not shown].) Four contacts are made between cellular CD4 and virion, three of which have led to activation. To sum up, there are four cellular CD4 attachment contacts and four activated SU-TM (although overlapping, not the identical sets of four). The result is infection. Enhancement has occurred: a virion that would not have infected in the absence of sCD4 can now infect. (g) There is 50% higher cell surface CD4 concentration than in panels e and f. Because of the higher SU density on the PI virion here than on the TCLA virion in panel d, six SU-CD4 contacts are now made. Four of these six lead to fusion activation: a fusion complex of four CD4-SU-TM can be formed. Infection that could not occur in panel e now results.

We suggest that the current occupancy model and its major rival, the few-hit theory of neutralization, can be put to crucial tests and their explanatory scopes can be compared. The degrees of neutralization achieved by neutralizing agents at concentrations around their dissociation constants agree better with the occupancy model than with the few-hit theories (Fig. 5). Kinetic data cannot differentiate between the rival hypotheses. However, our model can explain the PI neutralization resistance. But few-hit theories, all other things being equal, imply the inverse PI/TCLA neutralization sensitivity ratio, since a certain number of hits would constitute a lower occupancy on PI than TCLA virions.

Nevertheless, the testability of our model is problematic: at present it depends on the auxiliary hypothesis that differences in spike densities of total virion populations reflect such differences for the small infectious fractions of these populations. This is a formal problem both when different strains are compared and when the spike density of one strain is varied by mutagenesis or induced shedding of SU. However, there may be reason to reconsider the negligible ratios of infectious to noninfectious HIV-1 particles (20, 24). The inefficiency of HIV-1 adsorption (18) means that many of the noninfecting particles are nevertheless potentially infectious. Thus, physicochemical measurements on whole virion populations may yet prove informative about differences between infectious virion fractions. However, even further beyond reach of current experimental data is the concept of minimal SU number. Only when these factors become better known will the precise predictions of the model be testable. Such predictions are, e.g., that a small difference between the actual and maximal unoccupied SU numbers implies a minimal neutralizing concentration that is below the K_d for the interaction of the agent with the native oligomeric Env on virions. An actual SU number that is double the maximal number of unoccupied SU on a neutralized virion ($N = 2p$) implies that the minimal neutralizing concentration equals K_d. However, these predictions presuppose absence of competition and cooperation, which illustrates the potential complexity of neutralization.

Yet, aspects of the model are amenable to test: it implies that the variation in infectivity measured on different cell types could be due to distinct minimal SU numbers. Thus, a virus preparation could have different average relative amounts of spare SU in relation to different cell types. Neutralization resistance should be greatest in relation to the cell type for which the virus requires the lowest minimal SU number. Furthermore, the model may shed light on why neutralization sensitivity is affected both by which cells the virus is produced in and which cells are the target for infection (45). The producer cell effect would be accounted for by consequences on receptor affinity of differential Env glycosylation and by variations in the degree of incorporation of Env into virions. Any target cell effects may be explained by differential cell surface expression of receptors, which could yield distinct viral requirements for minimal numbers of SU.

A recombinant human IgG (IgG1b12) was recently produced by a phage display technique (7, 42). This MAb was shown potently to neutralize primary isolates, but the minimally neutralizing concentrations of this MAb are lower than those of other MAb for both TCLA and a number of PI (7). Thus, A_2/A_1 is high, just as for many other neutralizing antibodies. This is in line with the minimal relative occupancy determining neutralization potency. However, another MAb, 2F5, to the one known neutralization epitope on TM of HIV-1, also neutralizes PI less efficiently than TCLA (41). Although SU shedding decreases the affinity of 2F5 for virions (44), its epitope stays on the virion when SU is shed. How could resistance to neutralization by antibodies to this epitope then vary with the number of SU per virion? The answer may lie in the concept of functional Env heteromers or spikes. If the TM that have lost their SU are fusogenically inert (28), binding to them will have no biological effect. The number of spikes per virion that need to be inactivated by 2F5 would, therefore, still depend on the minimal and total spike number. The binding to inert TM will affect the binding to functional TM-SU complexes only negligibly, when the excess of antibody over antigen is vast.

Another phenomenon may also be covered by the same model: although blocking of an SU by sCD4 on the surface of the virion should theoretically be sufficient for rendering that SU inactive, the inducibility of dissociation of SU from TM correlates with the sensitivity to neutralization by sCD4 (16, 32, 36, 50). The explanation may be twofold. First, a nonneutralizing concentration of sCD4 can induce some shedding. Then after that event, a lower relative occupancy is required for neutralization—a relative occupancy now achieved by the initially nonneutralizing concentration. Second, when SU has been shed, cellular CD4 cannot compete with sCD4; neutralization is then irreversible. Certain MAbs also induce SU shedding (40). Their neutralization potency would also, according to the model, be influenced by the susceptibility of the virus to their induction of SU shedding. Induced shedding complicates the testability of the model: the relevant total SU number is the one that obtains at the minimally neutralizing conditions, when SU shedding may have occurred.

Both PI and TCLA adsorb poorly to CD4-positive cells, but they differ in their dependence on cell surface levels of CD4 for infection. CD4 is a limiting factor at higher levels of expression for PI than it is for TCLA (18). Still, neutralization that interferes with attachment to CD4 is much more efficient for TCLA, i.e., when cellular CD4 levels are not limiting, than when they are limiting, as for PI. Further modeling may resolve this seeming paradox: because of their lower spike density, TCLA may be incapable of using CD4 molecules above a certain cell surface concentration for multipoint attachment; their spike density may be too low to recruit cellular CD4 molecules above a certain CD4 density (Fig. 6). But because of the higher intrinsic affinity (or higher on-rate constant) of their oligomers for CD4, they may attach to the same extent as PI, which have weaker intrinsic binding of higher valency (36). The PI can be thought to have spare spikes, which may enhance infectivity, when receptor density is high (Fig. 6). An accessory receptor may become limiting for TCLA at lower cellular CD4 levels than for PI (18). Total and minimal numbers of SU-TM

oligomers and the other parameters and variables in the model may therefore all contribute to the phenotypic traits, including differential neutralization sensitivity, of PI and TCLA. The idea of a composite causation of the neutralization-resistant PI phenotype is supported by recent evidence of around 3-fold higher SU numbers on samples of PI than TCLA virions, around 10-fold lower affinity of neutralizing MAbs for oligomeric, cell surface-bound PI than TCLA Env, and over 100-fold greater relative resistance of PI than TCLA (50). However, a qualitative difference has also been discovered: PI but not TCLA infection is enhanced by sCD4 and MAbs at low concentrations (23, 46, 50). The concept of spare SU on PI virions may shed light on such a phenomenon. A mechanism can perhaps be found in differential triggering of fusion by CD4 (20), as we sketch in the iconic model in Fig. 6. There, a comprehensive explanation of the differential requirements of cellular CD4, neutralization sensitivity, and susceptibility to enhancement is delineated. Yet, other entities, which are not covered by the modeling, may exist. A better understanding of HIV-1 neutralization requires further dissection of the mechanisms of, and molecules involved in, virus attachment and entry, as well as improved knowledge of the structure and function of the Env protein (21, 38).

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REFERENCES

1. **Allaway, G. P., K. L. Davis-Bruno, G. A. Beaudry, E. B. Garcia, E. L. Wong, A. M. Ryder, K. W. Hasel, M.-C. Gauduin, R. A. Koup, J. S. McDougal, and P. J. Maddon.** 1995. Expression and characterization of CD4-IgG2, a novel heterotetramer that neutralizes primary HIV type 1 isolates. AIDS Res. Hum. Retroviruses **11:**533–539.
2. **Andrewes, C. H., and W. J. Elford.** 1933. Observations on anti-phage sera. I. "the percentage law." Br. J. Exp. Pathol. **14:**367–376.
3. **Ashkenazi, A., D. H. Smith, S. A. Marsters, L. Riddle, T. J. Gregory, D. D. Ho, and D. J. Capon.** 1991. Resistance of primary HIV-1 isolates to soluble CD4 is independent of CD4-gp120 binding affinity. Proc. Natl. Acad. Sci. USA **88:**7056–7060.
4. **Atkins, P. W.** 1986. Physical chemistry, 3rd ed., p. 687–712. Oxford University Press, New York.
5. **Baldinotti, F., D. Matteucci, P. Mazzetti, C. Giannelli, P. Bandecchi, F. Tozzini, and M. Bendinelli.** 1994. Serum neutralization of feline immunodeficiency virus is markedly dependent on passage history of the virus and host system. J. Virol. **68:**4572–4579.
6. **Bou-Habib, D. C., G. Roderiquez, T. Oravecz, P. W. Berman, P. Lusso, and M. A. Norcross.** 1994. Cryptic nature of envelope V3 region epitopes protects primary monocytotropic human immunodeficiency virus type 1 from antibody neutralization. J. Virol. **68:**6006–6013.
7. **Burton, D. R., J. Pyati, R. Koduri, S. J. Sharp, G. B. Thornton, P. W. H. I. Parren, L. S. W. Sawyer, R. M. Hendry, N. Dunlop, P. L. Nara, M. Lamacchia, E. Garratty, E. R. Stiehm, Y. J. Bryson, Y. Cao, J. P. Moore, D. D. Ho, and C. F. Barbas III.** 1994. Efficient neutralization of primary isolates of HIV-1 by a recombinant human monoclonal antibody. Science **266:**1024–1027.
8. **Cook, R. F., S. L. Berger, K. E. Rushlow, J. M. McManus, S. J. Cook, S. Harold, M. L. Raabe, R. C. Montelaro, and C. J. Issel.** 1995. Enhanced sensitivity to neutralizing antibodies in a variant of equine infectious anaemia virus linked to amino acid substitutions in the surface unit envelope glycoprotein. J. Virol. **69:**1493–1499.
9. **Creighton, T. E.** 1993. Proteins, 2nd ed. W. H. Freeman & Co., New York.
10. **Daar, E. S., X. L. Li, T. Moudgil, and D. D. Ho.** 1990. High concentrations of recombinant soluble CD4 are required to neutralize primary human immunodeficiency virus type 1 isolates. Proc. Natl. Acad. Sci. USA **87:**6574–6578.
11. **Della-Porta, A. J., and E. G. Westaway.** 1977. A multi-hit model for the neutralization of animal viruses. J. Gen. Virol. **38:**1–19.
12. **Dimmock, N. J.** 1993. The neutralization of animal viruses. Curr. Top. Microbiol. Immunol. **183:**71–73.
13. **Dulbecco, R., M. Vogt, and A. G. R. Strickland.** 1956. A study of the basic aspects of neutralization of two animal viruses, western equine encephalitis virus and poliomyelitis virus. Virology **2:**162–205.
14. **Eason, G., C. W. Coles, and G. Gettinby.** 1980. Mathematics and statistics for the biosciences. Ellis Horwood Limited, Chichester, England.
15. **Gelderblom, H. R.** 1991. Assembly and morphology of HIV: potential effect of structure on viral function. AIDS **5:**617–638.
16. **Groenink, M., J. P. Moore, S. Broersen, and H. Shuitemaker.** 1995. Equal levels of gp120 retention and neutralization resistance of phenotypically distinct primary human immunodeficiency virus type 1 variants upon soluble CD4 treatment. J. Virol. **69:**523–527.
17. **Icenogle, J., H. Shiwen, G. Duke, S. Gilbert, R. Rueckert, and J. Anderegg.** 1983. Neutralization of poliovirus by a monoclonal antibody: kinetics and stoichiometry. Virology **127:**412–425.
18. **Kabat, D., S. L. Kozak, K. Wehrly, and B. Chesebro.** 1994. Differences in CD4 dependence for infectivity of laboratory-adapted and primary patient isolates of human immunodeficiency virus type 1. J. Virol. **68:**2570–2577.
19. **Karush, F.** 1978. Affinity and the law of mass action, p. 181–197. *In* R. A. Good and S. B. Day (ed.), Comprehensive immunology. Plenum Press, New York.
20. **Klasse, P. J., and J. A. McKeating.** 1993. Soluble CD4- and CD4-immunoglobulin-selected HIV-1 variants: a phenotypic characterization. AIDS Res. Hum. Retroviruses **9:**595–604.
21. **Klasse, P. J., J. P. Moore, and B. A. Jameson.** 1993. The interplay of the HIV-1 envelope complex, gp120 and gp41, with CD4, p. 241–266. *In* W. J. W. Morrow and N. L. Haigwood (ed.), HIV molecular organization, pathogenicity and treatment. Elsevier, Amsterdam.
22. **Klasse, P. J., and Q. J. Sattentau.** Variants of HIV-1-LAI selected for the capacity to induce membrane fusion in the presence of a monoclonal antibody to domain 2 of CD4. AIDS Res. Hum. Retroviruses, in press.
23. **Kliks, S. C., T. Shioda, N. L. Haigwood, and J. A. Levy.** 1993. V3 variability can influence the ability of an antibody to neutralize or enhance infection by diverse strains of human immunodeficiency virus type 1. Proc. Natl. Acad. Sci. USA **90:**11518–11522.
24. **Layne, S. P., M. J. Merges, M. Dembo, J. L. Spouge, S. R. Conley, J. P. Moore, J. L. Raina, H. Renz, H. R. Gelderblom, and P. L. Nara.** 1992. Factors underlying spontaneous inactivation and susceptibility to neutralization of human immunodeficiency virus. Virology **189:**695–714.
25. **Layne, S. P., M. J. Merges, M. Dembo, J. L. Spouge, and P. L. Nara.** 1990. HIV requires multiple gp120 molecules for CD4-mediated infection. Nature (London) **346:**277–279.
26. **Layne, S. P., M. J. Merges, J. L. Spouge, M. Dembo, and P. L. Nara.** 1991. Blocking of human immunodeficiency virus infection depends on cell density and viral stock age. J. Virol. **65:**3293–3300.
27. **Layne, S. P., J. L. Spouge, and M. Dembo.** 1989. Quantifying the infectivity of human immunodeficiency virus. Proc. Natl. Acad. Sci. USA **86:**4644–4648.
28. **Marcon, L., and J. Sodroski.** 1994. gp120-independent fusion mediated by the human immunodeficiency virus type 1 gp41 envelope glycoprotein: a reassessment. J. Virol. **68:**1977–1982.
29. **McKeating, J. A., A. McKnight, and J. P. Moore.** 1991. Differential loss of envelope glycoprotein gp120 from virions of human immunodeficiency virus type 1 isolates: effects on infectivity and neutralization. J. Virol. **65:**852–860.
30. **McLain, L., and N. J. Dimmock.** 1994. Single-hit and multi-hit kinetics of immunoglobulin G neutralization of human immunodeficiency virus type 1 by monoclonal antibodies. J. Gen. Virol. **75:**1457–1460.
31. **Moore, J. P.** 1990. Simple methods for monitoring HIV-1 and HIV-2 gp120 binding to soluble CD4 by enzyme-linked immunosorbent assay: HIV-2 has a 25-fold lower affinity than HIV-1 for soluble CD4. AIDS **4:**297–305.
32. **Moore, J. P., L. C. Burkly, R. I. Connor, Y. Cao, R. Tizard, D. D. Ho, and R. A. Fisher.** 1993. Adaptation of two primary immunodeficiency virus type 1 isolates to growth in transformed T cell lines correlates with alterations in the responses of their envelope glycoproteins to soluble CD4. AIDS Res. Hum. Retroviruses **9:**529–539.
33. **Moore, J. P., Y. Cao, L. Qing, Q. J. Sattentau, J. Pyati, R. Koduri, J. Robinson, C. F. Barbas III, D. R. Burton, and D. D. Ho.** 1995. Primary isolates of human immunodeficiency virus type 1 are relatively resistant to neutralization by monoclonal antibodies to gp120 and their neutralization is not predicted by studies with monomeric gp120. J. Virol. **69:**101–109.
34. **Moore, J. P., and D. D. Ho.** 1995. HIV-1 neutralization: the consequences of viral adaptation to growth on transformed T cells. AIDS **9**(Suppl. A)**:**S117–S136.
35. **Moore, J. P., and P. J. Klasse.** 1991. Thermodynamic and kinetic analysis of sCD4 binding to HIV-1 virions and of gp120 dissociation. AIDS Res. Hum. Retroviruses **8:**443–450.
36. **Moore, J. P., J. A. McKeating, Y. Huang, A. Ashkenazi, and D. D. Ho.** 1992. Virions of primary human immunodeficiency virus type 1 isolates resistant to soluble CD4 (sCD4) binding and glycoprotein gp120 retention from sCD4-sensitive isolates. J. Virol. **66:**235–243.
37. **Moore, J. P., Q. J. Sattentau, P. J. Klasse, and L. C. Burkly.** 1992. A monoclonal antibody to CD4 domain 2 blocks soluble CD4-induced confor-

mational changes in the envelope glycoproteins of human immunodeficiency virus type 1 (HIV-1) and HIV-1 infection of $CD4^+$ cells. J. Virol. **66:**4784–4793.
38. **Moore, J. P., Q. J. Sattentau, R. Wyatt, and J. Sodroski.** 1994. Probing the structure of the glycoprotein gp120 of human immunodeficiency virus type 1 with a panel of monoclonal antibodies. J. Virol. **68:**469–484.
39. **O'Brien, W. A., S.-H. Mao, Y. Cao, and J. P. Moore.** 1994. Macrophage-tropic and T-cell line-adapted chimeric strains of human immunodeficiency virus type 1 differ in their susceptibilities to neutralization by soluble CD4 at different temperatures. J. Virol. **68:**5264–5269.
40. **Poignard, P., T. Fouts, D. Naniche, J. P. Moore, and Q. J. Sattentau.** Neutralizing antibodies to human immunodeficiency virus type 1 gp120 induce envelope glycoprotein subunit dissociation. J. Exp. Med., in press.
41. **Purtscher, M., A. Trkola, G. Gruber, A. Buchacher, R. Predl, F. Steindl, C. Tauer, R. Berger, N. Barrett, A. Jungbauer, and H. Katinger.** 1994. A broadly neutralizing human monoclonal antibody against gp41 of human immunodeficiency virus type 1. AIDS Res. Hum. Retroviruses **10:**1651–1658.
42. **Roben, P., J. P. Moore, M. Thali, J. Sodroski, C. F. Barbas III, and D. R. Burton.** 1994. Recognition properties of a panel of human recombinant Fab fragments to the CD4 binding site of gp120 that show differing abilities to neutralize human immunodeficiency virus type 1. J. Virol. **68:**4821–4828.
43. **Sattentau, Q. J., and J. P. Moore.** 1995. Human immunodeficiency virus type 1 neutralization is determined by epitope exposure on the glycoprotein gp120 oligomer. J. Exp. Med. **182:**185–196.
44. **Sattentau, Q. J., S. Zolla-Pazner, and P. Poignard.** 1995. Exposed and masked epitopes on functional, oligomeric gp41 molecules. Virology **206:**713–717.
45. **Sawyer, L. S., M. T. Wrin, L. Crawford-Miksza, B. Potts, Y. Wu, P. A. Weber, R. D. Alfonso, and C. V. Hanson.** 1994. Neutralization sensitivity of human immunodeficiency virus type 1 is determined in part by the cell in which the virus is propagated. J. Virol. **68:**1342–1349.
46. **Schutten, M., A. C. Andeweg, M. L. Bosch, and A. D. M. E. Osterhaus.** 1995. Enhancement of infectivity of a non-syncytium inducing HIV-1 by sCD4 and by human antibodies that neutralize syncytium inducing HIV-1. Scand. J. Immunol. **41:**18–22.
47. **Smith, D. H., R. A. Byrn, S. A. Marsters, T. Gregory, J. E. Groopman, and D. J. Capon.** 1987. Blocking of HIV-1 infectivity by a soluble, secreted form of the CD4 antigen. Science **238:**1704–1707.
48. **Spouge, J. L.** 1994. Viral multiplicity of attachment and its implications for human immunodeficiency virus therapies. J. Virol. **68:**1782–1789, 1994.
49. **Stamatatos, L., A. Werner, and C. Cheng-Mayer.** 1994. Differential regulation of cellular tropism and sensitivity to soluble CD4 neutralization by the envelope gp120 of human immunodeficiency virus type 1. J. Virol. **68:**4973–4979.
50. **Sullivan, N., Y. Sun, J. Li, W. Hoffmann, and J. Sodroski.** 1995. Replicative function and neutralization sensitivity of envelope glycoproteins from primary and T cell line-passaged human immunodeficiency virus type 1 isolates. J. Virol. **69:**4413–4422.
51. **Taylor, H. P., S. J. Armstrong, and N. J. Dimmock.** 1987. Quantitative relationships between an influenza virus and neutralizing antibody. Virology **159:**288–298.
52. **Weiss, R. A., P. R. Clapham, J. N. Weber, A. G. Dalgleish, L. A. Lasky, and P. W. Berman.** 1986. Variable and conserved neutralisation antigens of human immunodeficiency virus. Nature (London) **324:**572–575.
53. **Wrin, T., T. P. Loh, J. C. Vennari, H. Schuitemaker, and J. H. Nunberg.** 1995. Adaptation to growth in the H9 cell line renders a primary isolate of human immunodeficiency virus type 1 sensitive to neutralization by vaccine sera. J. Virol. **69:**39–48.