Envelope V2 Configuration and HIV-1 Phenotype: Clarification

Recently we published a report on the role of the human immunodeficiency virus–type 1 (HIV-1) gp120 V2 domain in biological phenotype evolution (*1*). Chimeric viruses that contained either the V2 or the V3 domain of a syncytium-inducing (SI) virus exhibited intermediate SI capacity, whereas exchange of V2 and V3 together conferred a full SI phenotype. Sequence analysis revealed a hypervariable locus in the V2 domain that was elongated in SI and coexisting clones that did not induce syncytia (NSI), defined as switch-NSI clones. In contrast, NSI clones, obtained from patients with acquired immunodeficiency syndrome (AIDS) who had never developed SI clones, were defined as stable-NSI clones. These clones in general did not have elongated V2 domains. NSI HIV-1 biological clones with elongated V2 domains in four out of four patients preceded the emergence of HIV-1 variants with an SI phenotype (SI conversion). These observations lead us to speculate (*1*) that the elongation of the V2 domain might be used as a prognostic marker for future development of SI viruses.

In our follow-up study, polymerase chain reaction (PCR) analysis of DNA isolated from peripheral blood mononuclear cells from 11 patients obtained 11 to 60 months before SI conversion only showed the presence of elongated V2 domains in two cases (data not shown). In the first analysis (*1*), biologically cloned viruses were used. Because switch NSI viruses may be only a minor population in the total pool of viruses, the absence of elongated V2 domains could be a result of the limits of PCR detection in the bulk analysis. Given these findings, it seems unlikely that V2 length polymorphism can be used as a convenient prognostic marker for SI development, although our new data do not rule out a role for V2 in the development of an SI phenotype.

The association we originally reported between elongated V2 sequences in NSI clones and subsequent appearance of SI clones (*1*) suggested that an extended V2 domain for SI envelope conformation might be required. However, not all SI clones and associated NSI clones had elongated V2 sequences (*1*). Extension of the V2 domain may only transiently contribute to the "fitness" for transition from an NSI to an SI envelope conformation (*2*). Elongated V2 domains would then be present preferentially close to the moment of SI conversion. To distinguish between these two possibilities we enlarged our studies to a total of 37 persons carrying SI viruses. Twelve persons were studied within 3 months from the time of SI conversion, 25 persons were studied more than 6 months after SI conversion. In addition, we enlarged the group of stable-NSI viruses to a total of 55. In this latter group, 19 out of 55 (34%) had elongated V2 domains.

At SI conversion, 11 out of 12 (92%) individuals had viruses with elongated V2 domains. With respect to V2 elongation, switch-NSI viruses and SI viruses obtained at the moment of SI conversion were significantly different from the group of stable-NSI viruses ($P = 0.0007$, Fisher's exact test). However, in the group of switch-NSI and SI viruses obtained more than 6 months after SI conversion, only 14 out of 26 (54%) had elongated V2 domains. With respect to the length of the V2 domain, SI and switch-NSI viruses isolated late after SI conversion were not significantly different from stable-NSI viruses.

Our current data suggest that elongation of V2 may at least in a large fraction of cases be transiently required for SI conversion, but not for maintenance of the SI phenotype. This may explain why, in transsectional analyses on virus isolates, other groups have not been able to confirm out initial observation that the majority of SI viruses carry elongated V2 domains (*3*). Currently, we are analyzing whether SI clones with elongated V2 domains can be detected at the moment of SI conversion in patients from whom we isolated SI clones with short V2 domains from later time points. This will reveal whether viruses exist that go through SI conversion without V2 elongation.

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T Cell Receptor–MHC Class I Peptide Interactions: Affinity, Kinetics, and Specificity

Reversible macromolecule binding reactions are often assumed to follow a simple Langmuir kinetic pathway (*1*). This model, with the occasional addition of cooperativity and two or more types of binding sites, is generally adequate to describe equilibrium assay data (*2*). With the availability of surface plasmon resonance technology in the form of the BIAcore (Pharmacia Biosensor, Piscataway, New Jersey) instrument, the dynamics of binding reactions can be observed as they occur, offering the opportunity to obtain a clearer understanding of the kinetic pathway to binding.

Considerable information about binding dynamics is available through inspection of the signals from a well-designed BIAcore experiment. Our examination of the graphic and numerical data in figure 2 and table 1 of the report by Corr *et al.* (*3*) suggests that the model presented (two independent Langmuir systems) is inappropriate. We were unable to simulate this data (*3*) with a two independent site model, confirming what we had concluded by inspection. However, we think Corr *et al.* (*3*, p. 948) may be correct in stating that "The biphasic character of the binding curves . . . suggests that the binding of major histocompatibility complex (MHC)–peptide with immobilized T cell receptors (TCRs) is not a simple interaction but may require structural readjustment or conformational changes of the components." Thus they imply that a two-site model is comparable to a two-state model. A model with two states of a single bound complex is experimentally differentiable, as well as mathematically different, from a model with two types of bound complex.

To illustrate both the mathematical and experimental differences that distinguish these models, we simulated the three models: (i) a single-site Langmuir reaction, (ii) a reaction with two independent Langmuir subsystems, and (iii) a reaction in which the complex moves between two states.

A Langmuir reaction (Fig. 1A) contains just one type of ligand and one type of ligate. The ligand and ligate may be either separate or joined in one type of complex. Equation A describes this reaction (model 1).

Here (AB) is the concentration of complex, A is the concentration of ligand, B is the concentration of ligate, and RU is the measured signal. A reaction with two independent Langmuir subsystems (Fig. 1B) is one in which each subsystem follows the Langmuir equation independently. Equation B [model 2 and Corr *et al.* (*3*)], which describes this type of reaction, is simply the Langmuir equation repeated twice, with the amount of complex formed by each subsystem added to give the total signal.

A binding reaction with one ligate, one ligand, and a complex that can exist in one of two states [model 3, and discussion by Corr *et al.* (*3*)] describes binding that can include structural readjustment of the ligand or the ligate or both. Structural or conformational changes are evident by the increased stability, and hence the increased average half-life they give a complex. This reaction (Fig. 1C) is described mathematically by Equation C.

Here k_{conf} represents the characteristic time required for the complex to change to a generally more stable state. The term "multiphasic kinetics" is used to describe these last two models. These models are mathematically different, and describe differences that are experimentally observable.

The kinetic pathway described by each of these models is different, although the time progression of a single concentration of ligate may be fit quite well by two, or even all of these models. A ligate concentration series, however, generally contains enough information to discriminate between these models. There are two straightforward ways to determine if a model is reasonable. First, a look at an overlay plot of a concentration series often shows which models may be eliminated from consideration. Second, a reasonable model will have parameters that are constant (say, within three standard errors of each other) over the concentration series. When either of these tests fail, the description provided by the mathematics fails to catch the essence of the reaction, and parameters based on this description are questionable. The kinetic pathways of these three models can be graphed (Fig. 1).

In model 1 (a single Langmuir system), we see the classic kinetic pathway, a single exponential rise to an equilibrium value that is dependent on ligate concentration. There are about two orders of ligate magnitude between measurable binding and saturation, as expected from theory (*4*). For model 2 (a binding reaction with two independent Langmuir subsystems), complete saturation now requires almost four orders of ligate magnitude. There is a pseudo-saturation binding level reached about halfway through this concentration series, then a second rapid increase in binding with ligate concentration occurs, followed by true saturation. In model 3 (a single ligand-ligate system with two complex states), the pseudo-saturation characteristic or model 2 is missing. Here, there are two slopes evident during the ligate injection phase: a rapid increase (fast initial association), followed by a slower increase in the binding signal that results from the conversion of initially bound ligate to the final conformation.

Binding experiments that use the BIAcore instrument (*7*) are easily designed to allow visual examination of the data and so suggest an appropriate model [for example, (*5*, *6*)]. The injection phase of the experiment should cover a ligate concentration series of at least two, and preferably three, orders of magnitude. This provides enough data for comparison with the above plots, and can suggest which type of kinetic pathway the reaction follows. Once a model is selected, and consistent parameter estimates are obtained over the entire concentration series, the model may be accepted as a description of the reaction (*8*).

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A

$$A + B \Leftrightarrow (AB) \left\{ \frac{d(AB)}{dt} = K_{on}[B - (AB)] - k_{off}(AB) \right\}$$

$$RU = k_{RU}(AB)$$

B

$$A + B \Leftrightarrow (AB) \qquad \left(\begin{bmatrix} \frac{d(AB)}{dt} \\ \frac{d(AC)}{dt} \end{bmatrix} = \begin{Bmatrix} k(AB)_{on}[B - (AB)]A - k(AB)_{off}(AB) \\ k(AC)_{on}[C - (AC)]A - k(AC)_{off}(AC) \end{Bmatrix} \right)$$

$$A + C \Leftrightarrow (AC)$$

$$RU = k_{RU}[(AB) + (AC)]$$

C

$$A + B \Leftrightarrow (AB) \Leftrightarrow (AB)' \left(\begin{bmatrix} \frac{d(AB)}{dt} \\ \frac{d(AB)'}{dt} \end{bmatrix} = \begin{Bmatrix} k_{on}(B - [AB] - (AB)']A - k_{off}(AB) - k_{conf}(AB) \\ k_{conf}(AB) - k'_{off}(AB)' \end{Bmatrix} \right)$$

$$RU = k_{RU}[(AB) + (AB)']$$

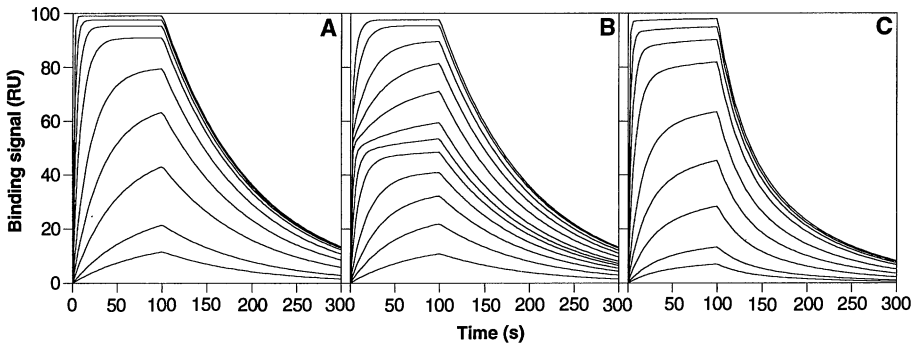

Fig. 1. Binding reaction simulations with three possible reaction models. Each simulation made under the assumptions of 100 RUs of immobilized binding sites and no material transport effects. (**A**) Simulation of a single Langmuir system with k_{on} = 1 RU^{-1} s^{-1} and k_{off} = 0.01 s^{-1}. (**B**) Simulation of two independent Langmuir systems with $k(AB)_{on}$ = 1 RU^{-1} s^{-1}, $k(AB)_{off}$ = 0.01 s^{-1}, $k(AC)_{on}$ = 0.01 RU^{-1} s^{-1}, $_{off}$ = 0.01 s^{-1}. (**C**) Two-state binding system with k_{on} = 1 RU^{-1} s^{-1}, k_{off} = 0.03 s^{-1}, k_{conf} = 0.01 s^{-1}, and k'_{off} = 0.01 s^{-1}. As the *RU* unit is related to concentration in g per liter, the molar units to which these parameters correspond depend on the molecular weights assumed for the ligand and ligate. Although individual flow concentrations give similar curves, each model has a characteristic behavior as flow concentration is varied.

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7. R. J. Fisher and M. Fivash, *Cur. Opin. Biotechnol.* **5**, 389 (1994). This paper contains a review of the current uses and analysis methods that employ surface plasmon resonance (mostly BIAcore) technology, and details several of the issues involved in the analysis of BIAcore data.
8. Several effects of the BIAcore experimental apparatus we have not discussed. An often significant effect is that of ligate delivery from the flowing solution to the immobilized ligand on the surface. A simple means of mathematically handling this effect is presented by Fisher *et al.* (6), however, the resulting equations must be solved numerically. Because material transport can be observed experimentally, an "easy" solution to this problem is to slow the binding reaction so that material transport is much faster than binding. Other machine effects are generally additive, and so can be subtracted from the measurements once they are understood.

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Response: We thank our colleagues for indicating that other mechanistic models might be consistent with our kinetic data (*1*) on the binding of sH-2L^d–peptide complexes to the purified, immobilized 2C TCR. It is well known that the mathematical descriptions of different models may often be used to effectively curve-fit the same data. (*2*), and statistical evaluation of such data and additional experimental verification are required to conclude that one model is more appropriate than another. In our report of the surface plasmon resonance (SPR) measurements of the TCR-MHC–peptide interaction (*1*), our intent was to exploit the SPR technology to estimate the specificity, kinetics, and affinity of the interaction, not to determine the precise molecular mechanism, which in this model system is a complex binding reaction involving the two chains of the T cell receptor, the two chains of the H-2L^d molecule, and the antigenic peptide. As a first consideration, we interpreted our kinetic association data in light of the simplest models: either a single site- or a two-site model, and from the data presented conclude that the second more closely fit the data. On the basis of curve fitting alone, we do not feel that the data are adequate to discriminate the two-sight model from the sequential two-state model described by our colleagues. For several immunological, biological, and biochemical reasons, such a conformational model is attractive, and thus we raised the possibility in our discussion, though we in no way intended to equate the two site model with a two state one. Because of intrinsic heterogeneities (that result from glycosylation, proportion of empty and peptide filled molecules, different orientation of immobilized molecules, multimers, and so forth) in our biologically active protein preparations derived from tissue culture cells, we are obligated to produce and examine more highly purified protein preparations to eliminate such heterogeneity as an explanation for our results. To conclude that the data support a two-state model in the light of such biochemical heterogeneity would not be appropriate. The criticism of Fisher and Fivash is apparently based on visual inspection of our published binding curves, not on a detailed evaluation of our data sets with statistical comparison of the relative validity of the fits to the different models.

In the discussion of mathematical models compatible with experimental binding data, our goal is to understand a biologically important set of binding reactions that initiate a cellular response. Any experimental or theoretical approach to this understanding is welcome, but any alternative mechanistic model must be further examined experimentally. The combination of several different independent experimental approaches to establish the consistency of any model is required. Such experiments should offer insight into the molecular intricacies of the TCR-MHC-peptide interaction.

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