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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17 January 1995; revised 3 February 1995; accepted 13 February 1995

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.