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M-2 peptide: A potential immuno-modulator of gp120 conformation

Anna Roitburd*, Gal Dela and Jonathan M Gershoni

Address: Department of Cell Research and Immunology, Tel Aviv University, Tel Aviv, Israel
* Corresponding author

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Background

Cellular CD4 induces HIV-1 gp120 into a unique conformation (CD4i) which is thought to expose previously concealed neutralizing gp120 epitopes. Therefore, CD4/gp120 complex has been considered as a potential HIV candidate vaccine. However, this complex is not without drawbacks. As part of an immuno-complex, CD4 might be expected to elicit an autoimmune response. Moreover, the CD4-induced conformation of gp120 is the result of occlusion of the CD4 binding site itself thus forfeiting a major neutralizing epitope of the envelope. In view of this, we sought to isolate gp120 binding peptides that stabilize gp120 in the CD4i conformation without occluding the CD4 binding site. Such peptides would function as immuno-modulators of gp120 conformation and could be useful in vaccine design. Therefore, through screening of a combinatorial phage display library comprised of random 12-mer cys-looped peptides, we succeeded in isolating a gp120451 specific peptide, designated m-1. The m-1 peptide locked gp120 into the CD4i conformation, as illustrated by the binding of the CD4i-mAb CG10. Moreover, m-1 was found not to occlude the CD4 binding site, as it continued to bind gp120 in the presence of soluble CD4. The m-1/gp120 complex was also found to maintain the integrity of other highly neutralizing epitopes such as that of the b12 mAb in addition to the CD4i epitopes. As such, the complex is regarded as a viable immunogen for vaccine development.

Objective

To develop a second generation peptide immuno-modulator based on the lead peptide m-1 with improved affinity for gp120 and expanded cross-reactivity for other HIV gp120 variants. For this we have combined the power of genetic engineering with the strength of combinatorial technology.

Results

In order to modify the m-1 peptide, a novel phage display library was constructed based on the m-1 gene using oligonucleotides which have undergone biased random mutagenesis. Briefly, this approach is based on the synthesis of corresponding oligonucleotides using phosphoramidite monomer precursors contaminated with regulated amounts of the other three possible phosphoramidites thus, generating a limited number of mutations in the synthesized sequence. Our «biased» library contained 4 × 108 mutant phage-displayed peptides, differing from the m-1 sequence by 3–4 amino acids/peptide and was subsequently screened against gp120451. A number of affinity purified phages were isolated that bound gp120 better than the original m-1 and were also improved CG10 inducers. To further select for improved gp120 binders, bio-panning was performed at high stringency conditions. In the process of doing so, a novel derivative of m-1 was selected. This phage-displayed peptide, designated m-2, had 10-fold improved binding to gp120. Moreover, m-2 was able to bind gp120s from various HIV strains (gp120BaL, gp120JRFL) in addition to gp120451.

Conclusion

Using genetic engineering and functional screening we have obtained the m-2 peptide, which effectively locks gp120 into a CD4i conformation, maintains the integrity

of the b12 epitope and retains a free CD4 binding site. In light of this, m-2 represents a new class of immuno-modulators for future AIDS vaccine design.