

Cross-Clade CD8⁺ T-cell Responses with a Preference for the Predominant Circulating Clade

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The genetic diversity of the human immunodeficiency virus (HIV) represents a major impediment toward a successful vaccine development. For example, the amino acid sequences of the 9 clades representing different phylogenetic groups of the HIV M sequences can differ by more than 10% and 30% within and between clades, respectively [1]. The distribution of these specific clades was thought to be a result of founder events where the virus was initially introduced and eventually predominated within a geographical area. However, global travel results in the introduction of new strain across geographical areas and further complicates the genetic diversity. Therefore, even if a vaccine is proven effective for a specific clade, it is uncertain if the protection extends to other strains within the clade and across different clades. This raises a question of whether several vaccines are needed for each of the distinct viral variants.

Since CD8⁺ T-cells have been shown to play an important role in containing HIV infection [2-6], vaccine development has been aimed towards the biological mechanism of CD8⁺ T-cell responses upon HIV infection [7]. Cross-clade CD8⁺ T-cell responses are affected by, among other factors, the viral proteins. Therefore one of the obvious strategies is to develop quantitative assays to assess the cross-reactivity across a large population of viral strains. The CD8⁺ T-cell responses to HIV-1 clades A, B, C and D gp160 (Env) were screened in 74 individuals using recombinant vaccinia-based interferon gamma (IFN- γ) Elispot assays [8]. The CD8⁺ T-cell responses are detected presumably as a result of immediate intracellular antigen expression, processing, and class I human leukocyte antigen (HLA) presentation.

Peripheral blood mononuclear cells (PBMCs) were isolated from fresh blood by gradient-centrifugation, and infected with recombinant clade A Env-vaccinia (vp1489), clade B Env-vaccinia (vp1198), clade C Env-vaccinia (vp1488), clade D Env-vaccinia (vt235), or wild-type Western Reserve vaccinia (ATCC VR 1354, negative control), or were stimulated with *Staphylococcus aureus* enterotoxin B (SEB, positive control). Overnight Elispot assays were performed [9] and dried plates were counted on an automated Elispot reader (Autoimmune Diagnostica). A positive assay was defined as

at least two times the negative control, at least 50 spot-forming units (SFUs) per million PBMCs after background subtraction, and a positive response to SEB.

Epitope mapping experiments were carried out using an HIV Env library comprised of 158 peptides that are 15 mers overlapping by 10 amino acids, representing the sequence of clade A. The peptide library was synthesized at Sigma Genosys using the PEPscreen process. Peptide Elispot assays were performed as described previously, using a peptide concentration of 3 $\mu\text{g/mL}$, with media as negative control, and individual peptide responses were confirmed in separate Elispot assays. Statistical analyses were used to examine the relation between response magnitudes between clades, to compare individual clade response frequencies, and to compare the magnitude of Elispot responses.

This study showed that 64% (47 of 74) women recognized at least one clade and 81% (38 of 47) responded to at least 2 of four clades (Table 1). These results indicate that cross clade CD8⁺ T-cell responses are common in the population tested, although a number of individuals have Env-specific CD8⁺ responses limited to clade A, the presumed infecting clade. In addition to clade A being the more frequently targeted, responses to clade A are stronger in a proportion of individuals. This might have significant implications in vaccine development since the manner by which each vaccine responds to diversity of the viral strains will eventually determine how a population is able to resist the challenge of diverse HIV strains.

Table 1. Frequency of Elispot responses to multiple clades of HIV-1 Env

No. of clades recognized in IFN- γ Elispot assays	No. of patients with positive Elispot responses (% of total responders)
1 clade	9/47 (19%)
2 clades	11/47 (23%)
3 clades	13/47 (28%)
4 clades	14/47 (30%)

While the CD8⁺ T-cell responses were considered reliable and unbiased, it was necessary to define the targets of these responses with precision. Epitope mapping studies were conducted by using an overlapping peptide library of autologous sequences to the clade A Env recombinant vaccinia. A subset of eight randomly selected women was chosen based on sample availability. The epitope-specific responses showed amino acid homology of the epitope regions between clade A and clades B, C, and D ranged from 56% to 100%, with an average homology of greater than 90%. The homology of these sequences is greater than the 70% homology of the entire gp160 sequence for the same strains. These data strongly support the high amount of cross-clade reactivity previously observed in the recombinant vaccinia assays. The observation that a diverse protein sequence can nevertheless generate cross-reactive T-cell

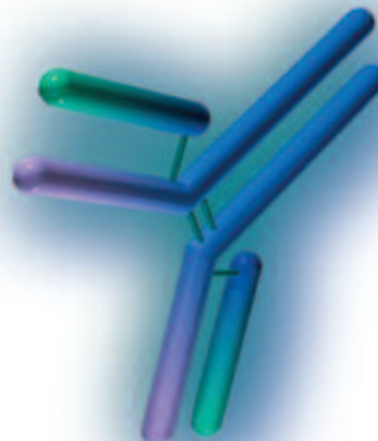
responses is an important consideration in developing vaccines targeted toward a diverse strain of viruses.

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