Genetic and Neutralization Sensitivity of Diverse HIV-1 env Clones from Chronically Infected Patients in China

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Background: HIV-1 continues to spread in China from traditional high-risk populations to the general public, and its genetic variability has become increasingly complex. However, the impact of these genetic changes on the biological and neutralization properties of the virus is unknown. The aim of this study is to generate full-length envelope molecular clones from chronically infected patients in China and characterize their genetic and neutralization properties against subtype-specific plasma pools as well as recently identified broadly neutralizing monoclonal antibodies (bnmAb) such as 2F5, 4E10, 2G12, PG9, PG16, lgG1b12 and VRC01.

Methods: PCR method was used to amplify the full-length envelope clones directly from patients’ PBMC and cloned into expression vector. Pseudotyped viruses built upon the viable envelope clones were used to study their biologic and immunologic features.

Results: A total of 107 full-length envelope molecular clones were obtained from 56 chronically infected patients in China. Phylogenetic analysis has shown that these viruses cluster tightly with reference sequences from CRF01_AE, subtype B', and subtype C/CRF07_BC/CRF8_B/C, with several clones having novel recombinant features. Pseudotyped viruses built upon the viable env clones have demonstrated their substantial variability in mediating viral entry, and in sensitivity to neutralization by subtype-specific plasma pools and bnmAb. In general, these viruses are sensitive to neutralization by subtype-specific plasma pools, although a subset of viruses either highly sensitive or resistant were also identified. In particular, we found that all viruses are sensitive to neutralization by 4E10 and scD4, but many are resistant to one or more bnmAb including recently identified PG9, PG16 and VRC01 known to recognize “vulnerable” sites on gp120 with exceedingly high neutralization activity against diverse viruses from outside China. Sequence and structural analysis has revealed several mechanisms by which the resistant viruses evade recognition by bnmAb including direct alteration in epitope sequence, shielding binding domains and so on.

Conclusions: We believe that the results presented here will help us to better understand the impact of genetic diversity on the neutralizing properties of the viruses, and to facilitate design of immunogens capable of eliciting antibodies with similar potency and breadth as bnmAb. It will also help to establish a broader Chinese virus panel to study the antibody response in infected and vaccinated individuals, and to contribute to the international virus panel which currently includes few Chinese viruses.

FIGURE 1: Unrooted neighbor-joining tree depicting the genetic relationships among the 107 full-length envelope molecular clones. The branch length is drawn by scale. The relationships between different sequences can be readily assessed. Individual sequences clustered with CRF01_AE are colored in green. Those clustered with subtype B' are shown in red, and those clustered with subtype CRF07_BC are shown in blue. A number of commonly used reference sequences for classifying HIV-1 subtypes and CRFs were also included and highlighted in each group with a different color. Branch colors represent these clones capable of mediating and entry, whereas those indicated by open circles failed to do so.

FIGURE 2: Identification of positive novel recombinants by SPOT analysis. Left: recombination pattern for the entire HIV-1 genome. The envelope genes analyzed include CNE1, CNE2, CNE3, CNE1 and CNE2 and were compared with pseudotype-IT, BC/CRF01_AE and BC/08-MF, as well as previously identified recombinant strains BC/019680 and BC/022115. Shown is a recombination pattern identified by phylogenetic analysis (Fig. 1). Right: Nucleotide position along the HIV-1 genome of the CNE1-YN2 and CNE2-YN2 clones. Shown is the percentage of identity within a sliding window of 96 bp, with a step size between plots of 48 bp. Comparison of these sequences was made against selected reference strains BC/019680/019680, BC/019680/019680, BC/019680/019680.

FIGURE 3: Spike-structural view of the interactions between gp120 of VRC01-resistant viruses and antibody VRC01. The interactions are shown as stick model, with light gray line and spheres. The heavy chains and light chains of antibodies VRC01 are shown in surface with green and orange colors, respectively. Residues highlighted in green in the heavy chains of VRC01 that also participate in binding is colored in yellow. Residues in the light chains of VRC01 that also participate in binding is colored in yellow. Residues in the light chains of VRC01 that also participate in binding is colored in yellow.

FIGURE 4: Neutralization sensitivity of selected HIV-1 isolates to subtype-specific plasma pools. The top 11 envelope clones capable of mediating viral entry were evaluated for neutralization sensitivity using the three subtype-specific plasma pools from chronically infected individuals. The average neutralization log10 titer for each plasma pool is indicated by a representative symbol. The black filled circles indicate the average log10 titer across all three plasma pools.