ABSTRACT

The generation of human monoclonal antibodies (mAbs) that are able to block HIV-1 infection *in vitro* would be useful reagents for studying virus neutralization, and assist in identifying neutralizing antibody (NAb) epitopes of HIV-1 envelope glycoprotein. This may provide important information for designing HIV-1 vaccine that aim to induce NAbs. HIV-1 subtype C individuals with high levels of NAb titres were identified, and peripheral blood mononuclear cells (PBMC) from these individuals were isolated and B-cells transformed with Epstein-Barr virus (EBV). Clones specific to HIV-1 gp120 using cell lysate preparations derived from HIV-1 subtype C infected cell lines were generated by performing limiting dilutions. Transformation efficiencies were estimated at over 80% by evaluating EBV-transformation cultures by microscopic visualization. Of these approximately 5% were HIV-1-specific. Five clones derived from the Du23 (1) sample secreting anti-HIV-1 antibodies were generated: 2.3C, 2.9D, 3.2C, 4.12E, and 1.5D. The 1.5D mAb could not be confirmed as anti-HIV-1 clone and it was probably lost during the process of subculturing. The remaining four Du23 mAbs were determined to be of IgG_1 isotype lambda (λ) light chain. These mAbs bind to gp120, and 2.9D is probably a polyreactive clone. Clones 2.3C, 3.2C and 4.12E appear to be A32-like, but do not share the same epitope. We have determined that the binding sites for all four Du23 mAbs require at least the C1 region, and they also showed binding sites overlapping with F91 and 1.5E. All four Du23 mAbs required intact gp120 proteins for their binding, and soluble CD4 enhance their binding. Thus, their binding site is discontinuous and conformational. These mAbs are non-neutralizing as they showed limited activity of 30-59% when tested using T-cell line grown viruses or 0-30% when tested against pseudovirions. This activity is rather low when compared to over 80% shown by broadly neutralizing mAbs that have been described in the literature. The challenge in generating mAbs, in particular subtype C-derived, is to find those antibodies capable of

suppressing viral replication *in vivo* and be capable of preventing infection. These reagents could be used to identify epitopes to guiding the design of HIV-1 subtype C envelope immunogens or vaccines. It is also envisaged that neutralizing antibodies used in therapeutic setting or in combination with antiviral drug therapy could reduce viral load and retard disease progression in infected people.