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Collection of phage–peptide probes for HIV-1 immunodominant loop-epitope

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Abstract

Early diagnosis and prevention of human immunodeficiency virus type-1 (HIV-1) infection, which remains a serious public health threat, is inhibited by the lack of reagents that elicit antiviral responses in the immune system. To create mimotopes (peptide models of epitopes) of the most immunodominant epitope, CSGKLIC, that occurs as a loop on the envelope gp41 glycoprotein and is a key participant in infection, we used phage-display technology involving biopanning of large random libraries with IgG of HIV-1-infected patients. Under the conditions used, library screening with IgG from patient serum was directed to the CSGKLIC epitope. Three rounds of selection converted a 12 mer library of 10^9 sequences into a population in which up to 79% of phage bore a family of CxxKxxC sequences ("x" designates a non-epitope amino acid). Twenty-one phage clones displaying the most frequently selected peptides were obtained and were shown to display the principal structural (sequence and conformational), antigenic and immunogenic features of the HIV-1 immunodominant loop-epitope. Notably, when the mixture of the phage mimotopes was injected into mice, it induced 2- to 3-fold higher titers of antibody to the HIV-1 epitope than could be induced from individual mimotopes. The described approach could be applicable for accurately reproducing HIV-1 epitope structural and immunological patterns by generation of specialized viral epitope libraries for use in diagnosis and therapy.

Keywords: Biopanning; Epitopes; gp41; HIV-1; Mimotopes; Phage-display

1. Introduction

Presently, HIV continues to spread over the continents, and has become the most dangerous slowly progressing pandemic threat (Rambaut et al., 2004; UNAIDS, 2005). No prevention has been found so far (McMichael, 2006) because the virus has the unique property of escaping both natural and laboratory-created prevention and therapeutic measures (Altman and Feinberg, 2004). Of primary concern in the efforts to developing effective protection against HIV is the lack of peptide substitutes of immunodominant epitopes to reproduce the crucial antigenic and immunogenic viral properties in experiments and trials. One approach has been the use of sequences of HIV epitopes to synthesize peptides using chemical methods. In fact, synthetic peptides representing viral epitopes are widely produced and used in diagnosis (Gnann et al., 1987) and in experiments on inhibition of viral entry into the target cell (Eckert and Kim, 2001). The major deficiency of these peptides is that their conformations are not stable, so they are non-homogeneous, tend to form aggregates, and, in general, do not display the natural epitope structure–function pattern correctly (Oldstone et al., 1991; Pan Chan Du et al., 2002). Another approach, is to allow the anti-HIV-1 antibody itself to recognize and select perfect peptides from vast phage epitope libraries (Parmley and Smith, 1988; Scott and Smith, 1990; Smith and Petrenko, 1997; see reviews Deroo and Muller, 2001; Gazarian,

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2005), displaying up to 10^{10} linear or constrained peptide sequences (Scholle et al., 2005). Currently, phage display is a rapidly progressing biotechnology that allows presentation and reproduction of diverse panels of engineered peptides and proteins. Their screening (*biopanning*: Parmley and Smith, 1988) retrieves collections of peptide substitutes of ligands of functional proteins (Kay and Hamilton, 2001), antibodies in particular, and peptide probes for detection of biological treatment agents (Petrenko and Vodyanoy, 2003). The methodology combines many technical elements of current protein and peptide expression and modification methods with simple microbiological procedure of fast amplification of hundreds of selected peptides.

Despite the advantages that the phage display offers for many systems, there is still a dearth of publications dealing with the selection of peptides for HIV-1 (Scala et al., 1999; Enshell-Seijffers et al., 2001). One reason for this is that HIV-positive serum, which is rich in virus-specific antibodies useful for isolation of valuable peptides, is inconvenient for successful selection due to the heterogeneity and variability of the antibody species in individuals, so that peptides for epitopes of special interest are only isolated by chance. The purpose of our experiments was to develop conditions for the high-yield selection of peptides for one particular epitope, the HIV-1 immunodominant (ID) epitope $_{603}$CSGKLIC$_{609}$ presented as a loop on the apex of the gp41 protein domain interacting tightly with gp120, with which the gp41 forms the heterotrimeric complex involved in the infection process.

In earlier attempts to isolate HIV-1-specific peptides, serum samples of two HIV-1(+) non-symptomatic patients were used to screen m13 gp8 libraries but no gp41 ID mimotope containing two cysteines to adopt the loop configuration similar to the epitope were isolated (Scala et al., 1999). In a second study, the screening of a 12 mer constrained library yielded three 5 mer peptides with CSGKLIC-like sequences (Enshell-Seijffers et al., 2001; peptides shorter than are designed for the library arise sometimes during construction as a result of deletions). To improve the results of mimotopes selection for this particular epitope, we performed a total of 10 biopanning experiments involving different gp3 peptide libraries and a panel of sera from patients with disease progression which at the time of the study initiated the multidrug antiretroviral therapy (HAART: highly active antiretroviral therapy); we determined the library parameters and the titer of serum antibody to the epitope ensuring large-scale and highly reproducible selection of peptides to the gp41 epitope.

2. Materials and methods

2.1. Serum and IgG

The donors of HIV-1-infected serum were four patients (designated Pt: 1 to 4) of the "Hospital General No. 1 Gabriel Mancera, IMSS", Mexico City, infected with B-subtype HIV-1 circulating in Mexico and were at the stage of disease progression requiring HAART. Viral load (RNA copies/ml) and CD4 counts (cells/mm^3) prior to and after 12–15 weeks of the therapy were as follows: Pt 1=30,400/277 and 51,600/315; Pt 2=24,700/267 and 20,300/239; Pt 3=116,000/26 and 5840/136; Pt 4=750,000/42 and 119,000/169. The drug combinations for HAART were: Pt 1 and 2: nucleoside ZDV (zidovudine) and ddC (zalcitabine)+non-nucleoside EFV (efavirenz); Pt 3 and Pt 4, ZDV, ddC with protease inhibitor NFV (nelfinavir). Serum was prepared from the blood drawn in the hospital and stored at −20 °C. The IgG fraction was isolated by single-step affinity purification with protein G-Agarose (Life Technologies, USA) (Gazarian et al., 2000) and stored in aliquots at −20 °C (yield of IgG was 80–85% taking its amount in serum as 8–10 mg/ml). Protein in the preparations was quantified by the Bradford assay and could include some non-IgG serum components.

2.2. Overlapping synthetic peptides

Peptides (6354) VLAVERYLKDQQLLG, (6355) ERYLKD QQLLGFWGC, (6356) KDQQLLGFWGCSGKL, (6357) LLG FWGCSGKLICTT, (6358) WGCSGKLICTTTVPW), (6359) GKLICTTTVPWNASW, were from the NIH AIDS Research and Reference Reagent Program.

2.3. Phage-display libraries

Peptide libraries, 12 mer linear, and 7 mer linear and constrained, were from New England BioLabs Inc. (Beverly, MA, USA). In each of them, random peptides (approx. 2.7×10^9 electroporated sequences) are fused to a minor coat protein (gp3) of m13 phage and expressed at its N-terminus separated by a Gly-Gly-Gly-Ser spacer. The libraries were amplified once to have 55 to 200 copies of each sequence per 10 μl, the amount used in single biopanning experiment.

2.4. Peptide selection and analysis

A standard biopanning procedure (Smith and Scott, 1993; Dower and Cwirla, 1994; Smith and Petrenko, 1997) was used with minor adjustments (Gazarian et al., 2001). Briefly, two wells in a 96-well polystyrene microtiter plate (Immulon 4 flat-bottom plates, Dynatech Lab Inc., USA) were coated with affinity purified IgG from each patient's serum. For this, one well was filled with 15 μg, the second with 7.5 μg of IgG, each in 100 μl of phosphate-buffered saline (PBS), and the plates were incubated overnight at 4 °C with gentle rocking. Unbound IgG was removed and the wells were washed 6 times with PBS-T (PBS–0.1% Tween 20) and blocked 1h at 4 °C with blocking buffer (PBS–1% BSA), followed by 5 consecutive washing steps using PBS-T. For affinity selection, 10 μl of the library (2×10^{10} plaque-forming units, PFU) were added to 190 μl of PBS-T and the mixture was distributed in the wells coated with IgG (100 μl/well); the wells were incubated for 1 h at room temperature (RT) with gentle rocking to allow phage to bind. The unbound phage were pipetted out and wells were washed 10 times with PBS-T (RT). Bound phage were eluted from each well by stirring with 100 μl of elution buffer (0.1 N HCl–glycine, pH 2.2). Eluted phage (designated further as "eluate") from two wells were combined and quickly neutralized by the addition of 2 M Tris (pH 9.1).