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Construction, purification, and immunogenicity of recombinant cystein–cystein type chemokine receptor 5 vaccine ☆

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Abstract

Cystein–Cystein type chemokine receptor 5 (CCR5) is a seven-transmembrane, G-protein coupled receptor. It is a major coreceptor with CD4 glycoprotein mediating cellular entry of CCR5 strains of HIV-1. A lack of cell-surface expression of CCR5 found in the homozygous Δ32 CCR5 mutation, upregulation of CC chemokines and antibodies to CCR5 are associated with resistance to HIV infection. In addition, CCR5 can be blocked by three CC chemokines and antibodies to three extracellular domains of CCR5. Consequently, CCR5 is considered an attractive therapeutic target against HIV infection. In the current study, we constructed a recombinant vaccine by coupling a T helper epitope AKFVAAWTLKAA (PADRE) to the N terminus of CCR5 extracellular domains (PADRE-CCR5) and expressed this protein in *Escherichia coli*. We have developed an inexpensive and scalable purification process for the fusion protein from inclusion bodies and the final yields of 6 mg purified fusion protein per gram of cell paste was obtained. The immunogenicity of the recombinant vaccine generated was examined in BALB/c mice. Sera from the vaccinated mice demonstrated high-titer specific antibodies to the recombinant vaccine, suggesting that PADRE-rCCR5 may be used as a candidate of active CCR5 vaccine.

Keywords: CCR5; PADRE; Peptide vaccine; Protein purification

Chemokine receptor 5 (CCR5)[1] is a G-protein coupled molecule that is expressed on T cells, macrophages, and immature dendritic cells [1–3]. CCR5 contains 352 amino acids with a calculated molecular mass of 40.6 kDa and shares 71% sequence identity with CCR2, with most of the differences being located on the extracellular and cytoplasmic domains [4–6]. CCR5 composed of seven hydrophobic transmembrane domains with an extracellular N-terminal segment and a cytoplasmic C-terminal tail containing structural motifs, which are critical for ligand-dependent signaling, desensitization, and receptor trafficking [7]. CCR5 contains four extracellular domains and a conserved DRYLAVHA sequence in the second intracellular loop, which has been implicated in G-protein interaction [7].

CCR5 is a coreceptor playing an important physiological role in regulating the movement of mononuclear cells by interacting with CC chemokine receptor for MIP-1α, MIP-1β, and RANTES [3,8,9]. It also serves as the main coreceptor for the entry of R5 strains of human immunodeficiency virus (HIV-1, HIV-2) [10]. In individuals infected with HIV-1, CCR5-tropic viruses are the predominant species isolated during the early stages of viral infection [11], and at least half of all infected individuals harbor only R5 viruses throughout the course of infection [12]. Genetic studies of a defective CCR5 allele (Δ32) have indicated that

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[1] *Abbreviations used:* CCR5, Cystein–Cystein type chemokine receptor 5; HIV-1, HIV-2, human immunodeficiency virus; PADRE, pan DR helper epitope; LB, Luria–Bertani.

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homozygous individuals are strongly resistant to HIV-1 infection and that heterozygotes have delayed progression to AIDS [13–16]. CCR5 is genetically stable, unlike viral targets, which may rapidly mutate during the course of infection. Consequently, CCR5 may be an antiviral therapeutic target. Some intervention strategies that attempt to inhibit viral replication by directly decreasing CCR5 expression have been investigated, including chemokines and their analogs, small molecular inhibitors, and small interfering RNAs [17–19]. PRO 140, an anti-CCR5 monoclonal antibody, potently inhibited HIV-1 replication in both peripheral blood mononuclear cells and primary macrophages [20]. However, antibody therapy is expensive and has to be repeatedly administered for long periods of time. An active vaccination strategy to induce anti-CCR5 antibodies that can bind the native CCR5 and block viral infection may be an alternative to passive infusional therapy.

A potent universal immunostimulant, known as pan DR helper epitope (PADRE), has been discovered that could enable the development of synthetic vaccines [21]. Immune responses stimulated with the PADRE synthetic vaccine exhibited hallmarks of protective immunity, with high titers of antibody consisting mainly of IgG, which persist longer than 12 months.

We fused PADRE (AKFVAAWTLKAA) to the N terminus of four extracellular domains of CCR5 (PADRE-rCCR5) and expressed this protein in *Escherichia coli*. The fusion protein formed inclusion bodies. We developed a strategy for purification of PADRE-rCCR5 from inclusion bodies. The final yields of 6 mg purified and refolded PADRE-rCCR5 per gram of cell paste can be obtained. BALB/c mice were immunized with the recombinant vaccine. Sera from the vaccinated mice showed high-titer specific antibodies against the recombinant vaccine.

Materials and methods

Materials and reagents

Restriction endonucleases, *Taq* polymerase, and T4 DNA ligase were purchased from Takara (Dalian, China); Urea, phenylmethylsulphonyl fluoride (PMSF) and β-mercaptoethanol, Triton X-100, and Tris were purchased from Serva (Germany); Sephacryl S-300 resin was purchased from Amersham Pharmacia Biotech (USA); 5-L Fermentor was purchased from B. Braun (Germany); Mouse anti-human CCR5 monoclonal antibody was purchased from R&D (USA); Goat anti-mouse IgG FITC conjugate, FBS, and RPMI 1640 medium were purchased from Sigma (USA). The plasmid pBV220 containing the P_R and P_L promoters, the clts857 gene, and two strong transcription terminators was constructed in-house [22]. All other chemicals were of analytical grade. Healthy female BALB/c mice (7 weeks old) were purchased from the National Rodent Laboratory Animal Resource, Shanghai, China.

Construction of PADRE-rCCR5 expression vector

DNA sequences coding for PADRE[21] and four extracellular domains of CCR5 (amino acid residues: 1–31, 89–102, 168–197, and 258–279) [23] based on the published nucleotide sequence (GenBank Accession No. U54994). The sequence of whole recombinant gene was designed to include the *Eco*RI, *Bam*HI, and *Pst*I restriction sites. The sequence from *Eco*RI site to *Bam*HI site is PADRE sequence. The four extracellular domains of CCR5 were linked by three soft linker GGGGS. The synthesis and cloning of the recombinant gene (PADRE was coupled to the N terminus of CCR5 extracellular domains) were done by Shenggong (Shanhai, China). The recombinant gene was subcloned into the sites of *Eco*RI and *Pst*I of the plasmid pBV220. The recombinant plasmid was identified using DNA sequencing and named pBV220-PADRE-rCCR5 (Fig. 1). *E. coli* DH5α cells were transformed with the plasmid pBV220-PADRE-rCCR5.

Expression of PADRE-rCCR5 in E. coli and fermentation

A single transformed DH5α colony was used to inoculate 10 ml Luria–Bertani (LB) medium supplemented with ampicillin (100 μg/ml) grown with 200 rpm shaking overnight at 30 °C. Three milliliters of culture was transferred to 300 ml fresh LB medium in a 500 ml shake flask. The culture was grown with 200 rpm shaking at 30 °C until the OD_{600} reached 0.5 and induced by changing temperature from 30 to 42 °C. After incubation at 42 °C for 4 h, 1 ml of culture was collected, analyzed by electrophoresis on a 15% polyacrylamide/sodium dodecyl sulfate gel, and stained by Coomassie blue R-250.

Fermentation was performed using the fermentor (working volume of 5 L). A single transformed colony was inoculated in 10 ml LB medium supplemented with ampicillin (100 μg/ml) and grown at 200 rpm and 30 °C overnight. Two hundred milliliters of semi-defined medium (16 g/L

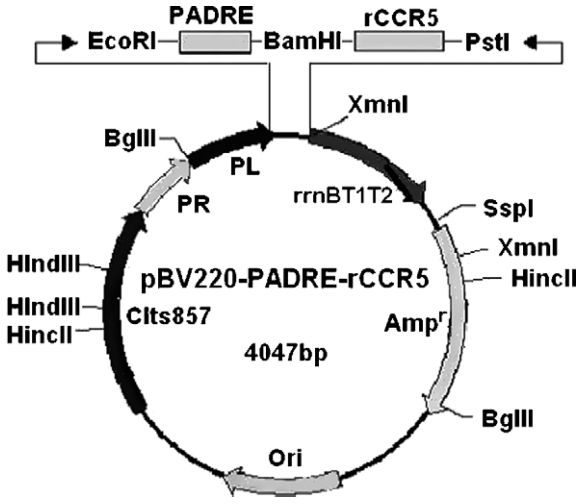

Fig. 1. Map of pBV220-PADRE-rCCR5 expression vector.