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Development of an immunoassay for differentiating human immunodeficiency virus infections — from vaccine-induced immune response in Tiantan vaccine trials in China

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ABSTRACT

Objective: To develop a Rapid Flow-through assay for distinguishing replicating Tiantan vaccine-generated serological response from true HIV infection.

Design and method: A Rapid Flow-through Test including gp41, gp36, sk1, sk2 and sk3 antigens was established and the performance of the assay was evaluated in clinical studies and compared with ELISA assay.

Results: Sk1, sk2 and sk3 peptides performed at 100% specificity and slightly but not significantly different sensitivities between ELISA assay (92%, 76% and 41%) and Flow-through Test Kit (92%, 75% and 40%) in diagnosing HIV-1 infections. Of particular importance, Tiantan vaccine recipients that gave false-positive results in gp41 serodetection scored negative for sk1, sk2 and sk3 antibodies.

Conclusion: The Rapid Flow-through Test could be a robust tool in both diagnosing HIV-1/2 infections and differentiating between vaccines induced immunity and immunity resulting from natural exposure, thus serving as potential implementation in HIV vaccine trials.

Introduction

Human immunodeficiency virus (HIV) pandemic is one of the most serious challenges to public health across the world. More than 60 million people have been infected with the virus, and more than 25 million people have died since HIV was discovered in 1983 [1]. In 2007, an estimated 2.7 million people became newly HIV infected and about 2 million people died of AIDS [2]. An HIV vaccine is the best hope in the long term for conquering AIDS [3]. Approximately 60 phase I/II clinical trials testing more than 30 HIV candidate vaccines have been completed over the last three decades [4] and at the present time, overall 200 HIV vaccine trials are undergoing across the world [5]. In 2007, Tiantan vaccine was allowed into a phase I vaccine trial in PR China. The vaccine, developed by the Chinese Center for Disease Control and Prevention, uses an attenuated, replicating Tiantan vaccinia vector to express multiple HIV immunogenic proteins (*gag, pol, nef and env*) of HIV-1 97CN54 (CRF-07), a strain of virus isolated from the Xingjiang Uygur Autonomous Region of the People's Republic of China [6,7]. As the only replicative vaccines in ongoing clinical trials throughout the world, Tiantan vaccine has demonstrated favorable safety and efficacy profiles and will soon enter phase II clinical trials [8].

It is well documented that many HIV vaccine recipients might react positively in commercial diagnostic assays that detect HIV antibodies, generating patterns indistinguishable from those for HIV-infected individuals [9]. This is primarily because most of the current HIV vaccine candidates contain antigenic viral components that are also included in HIV serodetection assays. The vaccine-induced false diagnosis has a significant impact on future clinical vaccine research. At first, the positive reaction with HIV detection assays complicate subsequent preventive vaccine trials, in which early detection of HIV infections is of critical importance. Secondly, the consequent socioeconomic harm to vaccine recipients, such as denied employment, health insurance, travel, immigration, could deter potential trial participants and severely curtail recruitment into further large-scale trials. By using a whole-HIV-genome phage display library, three antigenic HIV sequences in *Env* gp41 (designated sk1, sk2) and *Gag* p6 (designated sk3) were identified that are highly conserved among HIV clades and subtypes but not included in HIV vaccine

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candidates tested today [10]. In clinical trials involving 3000 infected patients with diverse HIV subtypes and 502 uninfected volunteers immunized by four non-replicating HIV vaccines ((HVTN 203, RV124, VRC004/006/009/010, VAX003/004) rather than Tiantan vaccine, the test kits using sk1, sk2 and sk3 antigens performed at 99% specificity and sensitivity in distinguish between non-replicating vaccine-induced antibodies and seroconversion due to true HIV infections [10,11].

In the current studies, we addressed the question whether serological test for sk1, sk2 and sk3 could serve as differential criteria for seropositive responses by replicative Tiantan vaccines and HIV infections. In the current studies, the performances of these short peptides were illustrated by ELISA assay. Then, a Flow-through Rapid Test Kit was established to differentiate Tiantan vaccine recipients from true HIV infections, thus serving as part of the HIV rapid detection algorithm in the future HIV vaccine trials.

Materials and methods

Reagents

Three sequences (sk1, sk2 and sk3) that do not contain known HIV-protective epitope peptides were chemically synthesized by Invitrogen, Shanghai, PR China. HIV recombinant proteins gp41 and gp36 have been previously described [12]. The amino acid sequences of sk1, sk2 and sk3 were shown in Table 1, according to Alamos database (http://hiv-web.lanl.gob). Sera panels (PRB205 and PRZ207) were purchased from SeraCare Life Sciences, USA. Western Blot Band Pattern and Abbott third-generation HIV-1,2,O Test Kit were from Bio-Rad Laboratories, USA and Abbott Laboratories, Canada, respectively.

Clinical serum samples

HIV-1/2 positive sera were from the Center for Disease Control of Sichuan Province, PR China. Post vaccine sera were from the Center for Disease Control of China, PR China. Serum samples of healthy subjects and individuals infected with hepatitis B virus (HBV), hepatitis C virus (HCV) and M. tuberculosis (TB) were provided by the Department of Clinical Laboratory, The Second Affiliated Hospital of Chongqing Medical University, Chongqing, PR China. All samples conducted in the studies were collected following the guidelines from the 2008 Helsinki-Ethical Principles for Medical Research Involving Human Subjects.

Analysis of HIV peptides by dot-blot

Nitrocellulose membranes were coated with sk1, sk2 and sk3 peptides (2.5 ng) and recombinant protein gp41 (0.3 ng). Blocked with phosphate buffered saline containing 5% milk powder and 0.05% Tween20 (MPBST), the membranes were incubated with serum samples (1:100 dilution in MPBST) and subsequently, horseradish peroxidase conjugated anti-human immunoglobulin (Beijing Zhongshan Biotech, PR China) for 2 h, respectively. The signals were visualized by the incubation with 0.1 M phosphate buffer (pH 7.5) containing 0.15 mg/mL diaminobenzidine (DAB). All the procedures were performed at room temperature and followed by extensive wash with 0.05% Tween20/phosphate buffered saline (pH 7.0), unless otherwise indicated.

Table 1
The amino acid sequence of three HIV-1 peptides.

Peptide	Amino acid sequence
sk1	LIAARIVELLGHSSLKGLRRGWEALKYLWNLLQYWGQELKNSAISL
sk2	AVAEGTDRVIEVVQRVCRAILNIPRRIRQGFERALL
sk3	SRPEPTAPPAESFRFGEEITPTPSQKQEPKDKELYPPLASLRSLFGNDPSSQ

Preparation of enzyme linked immunosorbent assay kits

96-well ELISA plates were coated with sk1, sk2 and sk3 (500 ng), sk1 + sk2 + sk3 peptide mixture (300 ng each) and recombinant protein gp41 and gp36 (200 ng), respectively. After a 2 h blocking with 0.5% BSA/PBST and extensive PBST wash, the coated plates were stored at 4 °C. The cut-off (CO) values of sk1, sk2, sk3, sk1 + sk2 + sk3, gp41 and gp36 were determined on the basis of the average absorbance value for 300 HIV negative sera (1:100 dilution in sample diluent buffer). For each of the peptide and recombinant antigens, the cut-off values were the average absorbance values for negative serum plus 5 standard deviations (SD). For sk1, sk2, sk3 and sk1 + sk2 + sk3, we considered HIV-1 seropositive if absorbance/cutoff ratio ≥ 1. In case of gp41, samples with absorbance/cutoff ratios ≥ 1 were considered an HIV-1 seropositive sample or Tiantan vaccine-induced serum sample. In case of gp36, samples with absorbance/cutoff ratios ≥ 1 indicated HIV-2 seropositive.

Evaluation of sensitivity and specificity of the ELISA kits

Serum samples from healthy subjects, Tiantan vaccinees, HIV-1/2-infected patients and individuals previously exposed to other infectious agents were collected to evaluate the performance of ELISA kits. 100 μL Sera, diluted in sample diluent buffer (Artron Bioresearch Inc, Canada) (1:100 dilution), was added to each well. After 30 min incubation at 37 °C and extensive PBST washes, HRP-conjugated goat anti-human IgG secondary antibody (1:15,000 dilution) added for 30 min. The bound conjugate detected with tetramethylbenzidine (TMB) and the resulting absorbance was measured at a 450-nm wavelength, using an enzyme immunoassay microplate reader (Thermo Labsystem MK3, America).

Preparation of the Flow-through Rapid Test Kit

Chemically synthesized peptides (sk1, sk2 and sk3) (5 μg), recombinant proteins gp41 and gp36 (0.3 μg), and goat anti-human immunoglobulin antibodies (1 μg) were deposited onto synthetic nitrocellulose (NC) membranes, respectively. The membrane was blocked with 3% (w/v) BSA/PBST (room temperature for 2 h), washed with PBST (15 min) and dried at 37 °C. The membrane was cut into circles, which were placed on the top of absorbent material within a plastic cartridge (Fig. 2-Model). The plastic cartridge, absorbent material and protein A colloidal gold conjugate solution were provided by Artron BioResearch Inc, Canada.

Evaluation of sensitivity and specificity of the Flow-through Rapid Test Kit

Serum samples from healthy subjects, Tiantan vaccinees, HIV-1/2-infected patients and individuals previously exposed to other infectious agents were collected to evaluate the performance of Flow-through Rapid Test Kit. 250 μL of sera (1:10 dilution in 0.01 M PBS) was added to sample wells of the test kit and after 30 s incubation with PBST (1500 μL per well), signals were visualized by addition of colloidal gold conjugate solution (Artron Bioresearch Inc, Canada).

Statistical analysis

The x^2 test was used to analyze our data and significant difference was considered if $P<0.05$.

Results

Confirmation of collected clinical serum samples

The results of licensed HIV serodetection assays for clinical samples were showed in Table 2. However, 27 (out of 30) Tiantan vaccine