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Development and preclinical safety evaluation of a new therapeutic HIV-1 vaccine based on 18 T-cell minimal epitope peptides applying a novel cationic adjuvant CAF01

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ABSTRACT

Therapeutic immunization of HIV-1-infected individuals with or without anti-retroviral therapy is a new promising disease prevention. To induce a new cytotoxic T_{CD8} lymphocyte (CTL) immunity during chronic HIV-1 infection 15 infrequently targeted but conserved HLA-supertype binding CTL epitopes from Gag, Pol, Nef, Env, Vpu and Vif were identified. The 15 T_{CD8} and three T_{CD4} helper peptides were GMP synthesised and formulated with a new adjuvant CAF01 which is a synthetic two-component liposomic adjuvant comprising the quaternary ammonium dimethyl-dioctadecyl-ammonium (DDA) and the immune modulator trehalose 6,6′-dibehenate (TDB). Using IFN-γ ELISPOT assay, T-cell immune induction by the vaccine was found to both CD4 and CD8 T-cell restricted peptides in HLA-A2 transgenic mice. Comprehensive toxicity studies of the CAF01 adjuvant-alone and together with different vaccines showed that CAF01 when tested at human dose levels was safe and well tolerated with only local inflammation at the site of injection and no systemic reactions. No pharmacological safety issues were observed in Beagle dogs. The HIV-1 vaccine toxicity study in the Göttingen Minipig® showed no systemic toxicity from five repetitive i.m. injections, each with a 2-week interval, of either the 18 HIV-1 peptide antigen solution (AFO18) or the AFO18–CAF01, in which the 18 HIV-1 peptides were formulated with the CAF01 adjuvant. Distinct inflammatory responses were observed in the injected muscles of the AFO18–CAF01 vaccine treated animals as a result of the immune stimulating effect of the adjuvant on the vaccine. The results of the toxicity studies provide optimism for phase I clinical trials evaluating the therapeutic HIV-1 T-cell vaccination approach using multiple subdominant minimal epitope peptides applying the novel cationic adjuvant CAF01.

1. Introduction

The HIV-1 infection does not confer lifelong immunity, but leads to breakdown of the immune system, opportunistic infections and death. The immunity induced by the infection itself can only partially control the HIV-1 infection [1]. Antiretroviral therapy (ART) can successfully inhibit the virus replication but cannot eradicate the virus [2]. Despite the success of ART, many patients particularly in low income countries are currently not being treated with ART, making the need for inexpensive prophylactic and/or therapeutic vaccines obvious [3,4].

Therapeutic immunization of HIV-1-infected individuals with or without ART is a new promising disease prevention [5,6]. The purpose of a targeted therapeutic vaccination is in addition to the existing immunity to induce a broader, more powerful and better directed immunity than the one induced by the HIV-1 infection. This would potentially modify the virus–host dynamic, lower the viral load and thus prolong the time to receiving ART and/or development of AIDS. A lower viral load in infected patients may further lead to lower spread in the population. It is hoped that immune targeting of HIV-1 infected cell reservoirs may even hold a potential for eradication of HIV-1 [5].

In order to induce a new cytotoxic T lymphocyte (CTL) immunity during chronic HIV-1 infection by therapeutic vaccination, multiple infrequently targeted but conserved HLA-A0201-restricted

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epitopes from the HIV-1 proteins Gag, Pol, Env, Vpu and Vif were identified [7,8]. These subdominant epitopes have previously been used in dendritic cell (DC) immunization of 12 healthy HIV-1 infected HLA-A2$^+$ individuals not in ART [9]. In this test-of-concept study it was observed that new cellular immunity could indeed be induced and directed towards such conserved areas independent of the viral load during the untreated infection. The observed weak antiviral effect of the new cellular immunity [9] could be explained by the limited numbers of DC produced and the limited amount of loaded peptides. The approach of isolation of autologous DC is furthermore costly and requires specialized laboratories, which is not feasible in most developing countries and, therefore, a new formulation of the therapeutic vaccine is needed.

The possibility of re-directing the immunity in HIV-1 infected healthy individuals encouraged to improve the immunization by using high peptide doses in combination with an adjuvant. Aluminium salts are by far the most widely used adjuvants in vaccines to date. Their mechanism of action is not yet fully understood. It is known, however, that they do not optimally support the development of Th1 cells, which are thought to play a major role in obtaining protective immunity against diseases such as HIV-1. This has prompted the development of new adjuvants.

The novel cationic CAF01 adjuvant has been shown capable of inducing a balanced Th1/antibody (Ab) immune response in several animal models using a wide range of antigens [10–12]. The CAF01 based vaccines also elicit a high degree of protection in animal models against diseases such as tuberculosis, influenza, malaria and chlamydia. CAF01 has been shown in tuberculosis subunit vaccine studies in animals to induce long-term protective immune responses characterized by high levels of persisting multifunctional T cells [13]. CAF01 has also been shown to facilitate the induction of multiple CD4 T cells responses, including subdominant CD4 T cells responses not seen during natural infection [14]. In relation to HIV, it was previously found in a mouse model that CAF01 in addition to its known immunological help in inducing CD4 T cell immunity also supported the functional activation of CD8 T cells using single or multiple minimal CTL epitope peptides [15].

The adjuvant CAF01 is a synthetic two-component liposomic adjuvant comprising the quaternary ammonium dimethyldioctadecyl-ammonium (DDA) and the immune modulator trehalose 6,6′-dibehenate (TDB), the latter being a synthetic analogue of mycobacterial cord factor trehalose 6,6′-dimycolate. CAF01 has currently been made available for clinical trials [16] with the adjuvant having an acceptable safety profile, as evaluated from several vaccine toxicity studies, some of which are described here.

It was therefore decided to use CAF01 as an adjuvant together with the new therapeutic HIV-1 vaccine. For that purpose, 15 CD8 T cell epitopes and two CD4 T cell epitopes conserved among HIV-1 strains of different subtypes were selected [7,8,17–19]. In addition, the universal CD4 T helper PADRE-peptide was included [20]. CD4 T cell help is needed for induction of new CD8 T cell immunity [21,22]; therefore three CD4 T cell epitopes are included in the vaccine. The CD4 epitopes and seven out of the CD8 epitopes were included in a previous therapeutic vaccine trial shown to be safe and well tolerated [9]. The15 CD8 T cell epitopes are predicted and/or confirmed to bind different the HLA supertypes A1, A2, A3, B7 and B44 [7,8,17] (Table 1). The use of HLA supertype restricted and conserved epitopes should theoretically cover more than 90% of any population [23]. The use of therapeutic vaccines with proteins or peptides in combination with adjuvants have been used in clinical trials against HIV-1 [24,25] and other chronic viral infections e.g. hepatitis C with promising results and no severe adverse effects [5,26].

The HIV-1 vaccine candidate AFO18–CAF01 was tested in mice and the results from the immunological mouse study and the subsequent pre-clinical safety evaluation in the Göttingen Minipig® are reported. Results from other CAF01 toxicity studies have been included as well. Based on the results from all the studies, it was considered safe to continue with the HIV-1 vaccine in phase I clinical trials.

2. Materials and methods

2.1. Peptide selection and GMP production

Fifteen CD8 T cell epitopes and two CD4 T cell epitopes conserved among HIV-1 strains of different subtypes were selected [7,8,17–19]. In addition, the universal CD4 T helper PADRE-peptide was included [20]. The epitope peptides comprised in the vaccine designated AFO18 are shown in Table 1. The peptides were manufactured in excess of 500 mg by C S Bio Co, CA, USA (GMP). Purity by HPLC was >95% and additional testing included mass spectrometry analysis, amino acid analysis, peptide content, water content, residual solvents, acetate content, endotoxin and bioburden, all with satisfactory results.

2.2. Mass spectrometry to identify peptides in the AFO18 peptide mix

Lyophilized peptide mixtures were dissolved in acetonitrile (MeCN)/0.1% trifluoro acetic acid (TFA). Samples were injected onto a C18 HPLC column (Acclaim pepmap100, LC Packings-Dionex, Amsterdam, NL) attached to a HPLC system (Ultimate, LC Packings-Dionex, Amsterdam, NL) and separated over a solvent gradient on the column. The separation was monitored by UV–vis at 214 and 265 nm immediately followed by MS analysis and MS^2 data dependent fragmentation on an iontrap mass spectrometer (Bruker Daltonics, Bremen, Germany). Data was analyzed with Hystar Post processing software (v3.1, Bruker Daltonics, Bremen, Germany) and Esquire Data analysis software (v3.3, Bruker Daltonics, Bremen, Germany).

2.3. CAF01 adjuvant production

The two adjuvant components DDA and TDB were synthetically manufactured by Avanti® Polar Lipids, AL, USA. The CAF01 adjuvant was manufactured at Statens Serum Institut (SSI) as previously described by the lipid film hydration method [12]. In brief, weighed amounts of DDA and TDB (5:1) were dissolved in chloroform/methanol (9:1, v/v) and the organic solvent subsequently removed by purging with a gentle stream of nitrogen, thereby forming a thin lipid film at the bottom of the flask. The lipid film was dried overnight under vacuum to remove trace amounts of the organic solvents. The lipid film was rehydrated in 10 mM Tris buffer (pH 7.4) by heating for 20 min at 60 °C with stirring to a final lipid concentration of 2.5 mg/mL DDA and 0.5 mg/mL TDB. This adjuvant formulation is denoted CAF01 2500/500. The adjuvant is stabile at 2–8 °C for 3 years.

2.4. Vaccine formulation

The adjuvanted vaccine is designated AFO18–CAF01, and includes all the 18 peptides formulated with the CAF01 adjuvant. In brief, the weighed amounts of each individual peptides were dissolved in 10 mM Tris buffer, pH 7.0 and the pH was adjusted to 7.0. The adjuvanted vaccine AFO18–CAF01 was formulated by mixing the peptide solution into the CAF01 adjuvant suspension. For the Göttingen Minipig® toxicity study, the AFO18 peptide solution (the peptides without adjuvant) was diluted to the appropriate concentration with Tris buffer, 10 mM, pH 7.0.