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Ex vivo production of autologous whole inactivated HIV-1 for clinical use in therapeutic vaccines

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ABSTRACT

This study provides a detailed description and characterization of the preparation of individualized lots of autologous heat inactivated HIV-1 virions used as immunogen in a clinical trial designed to test an autologous dendritic-cell-based therapeutic HIV-1 vaccine (Clinical Trial DCV-2, NCT00402142). For each participant, ex vivo isolation and expansion of primary virus were performed by co-culturing CD4-enriched PBMCs from the HIV-1-infected patient with PBMC from HIV-seronegative unrelated healthy volunteer donors. The viral supernatants were heat-inactivated and concentrated to obtain 1 mL of autologous immunogen, which was used to load autologous dendritic cells of each patient. High sequence homology was found between the inactivated virus immunogen and the HIV-1 circulating in plasma at the time of HIV-1 isolation. Immunogens contained up to 10^9 HIV-1 RNA copies/mL showed considerably reduced infectivity after heat inactivation (median of 5.6 $\log_{10}$), and were free of specified adventitious agents. The production of individualized lots of immunogen based on autologous inactivated HIV-1 virus fulfilling clinical-grade good manufacturing practice proved to be feasible, consistent with predetermined specifications, and safe for use in a clinical trial designed to test autologous dendritic cell-based therapeutic HIV-1 vaccine.

1. Introduction

Combined antiretroviral therapy (cART) inhibits HIV-1 replication to levels below the limit of detection of standard clinical assays, allowing the restoration of normal or nearly normal CD4 T cell counts and protective T cell immunity to opportunistic pathogens in most patients, and has dramatically reduced the morbidity and mortality of HIV-1 infection. However, in spite of these clinical benefits, cART is incapable of eradicating HIV-1, and also fails to restore HIV-specific T cell responses able to durably and effectively to control HIV-1 replication when antiretroviral drugs are discontinued [1–5]. Thus, cART must be administered for life, increasing the risk of drug-related adverse effects, the potential for emergence of drug resistant mutant viral variants, along with some inconvenience for the patients and economic burden for the patient and society [6,7].

Over the past several years, a variety of immune-based strategies including therapeutic vaccination and designed treatment interruptions have been explored as approaches that might allow effective host control of viral replication in the absence of continuous cART, but most of therapeutic vaccination studies to date have been disappointing, and some treatment interruption strategies have been associated with increased adverse events [8,9]. Nevertheless, the potential of an effective therapeutic vaccination approach is sufficiently attractive that such strategies continue to be evaluated, with dendritic cell-based anti-HIV-1 therapeutic vaccine strategies being of particular interest [10,11].

Myeloid DC are key cells for the generation and regulation of the adaptive immune responses by T cells. They are the most

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potent professional antigen-presenting cells, unique in their capacity to induce the de novo antigen-specific activation of naïve CD4 and CD8 T cells in vivo and in vitro (priming), as well as in their ability to present exogenous antigens, including pathogen derived peptides via MHC-class I molecules to stimulate responses by CD8 T cells, through processes called crosspriming and cross-presentation [12,13]. These extraordinary abilities make ex vivo generated antigen-loaded myeloid DCs an attractive and potent natural "cellular" adjuvant to induce desired T cell responses to antigens. This approach has been used in clinical trials in patients with cancer for more than a decade [14], and more recently it is also being explored in HIV-1/SIV infection.

Several studies performed in vitro and in vivo using animal models have demonstrated that autologous monocyte derived-dendritic cells (MDDCs) pulsed with HIV-1/SIV based immunogens can induce priming and boosting of virus specific immune response [15–19]. This approach has been also applied in infected patients by pulsing autologous MDDC with different kinds of immunogens: rgp160, HIV-1 peptides, canary pox vectors carrying HIV relevant genes, autologous HIV-1 RNA and autologous whole inactivated HIV-1, and, despite the different clinical study designs, the results suggest that the pulsed autologous MDDC immunizations were safe and well tolerated, could induce lymphoproliferation and virus-specific CD8 cell responses and, in some cases, transient virologic control [20–26].

Theoretically the use of whole inactivated HIV-1 as immunogen could have potential advantages over other HIV immunogens, including provision of the full complement of virion associated viral proteins, and depending on the method of inactivation, providing these virion proteins in their native conformation, which may be of particular importance for inducing desirable responses to conformational determinants on the viral envelope proteins. However, use of whole inactivated virions as a vaccine immunogen also has some potential drawbacks. Some of the viral proteins are not found in virions, while others are present in low amounts that may not confer optimal immunogenicity. In addition, the viral inactivation method applied must guarantee a high reduction of infectivity to assure safety, although this is less critical when using autologous virus for therapeutic vaccination compared to potential prophylactic immunization of seronegative subjects. Phase I/II clinical trials of therapeutic anti-HIV-1 vaccines based on autologous MDDC pulsed with autologous whole inactivated HIV-1 performed by Lu et al. [25] and our group [26], have demonstrated the safety of this approach and its ability to induce HIV-1 specific cellular immune responses, with a suggestion of antiviral activity in the Lu et al. study. Differences between the results of Lu et al. and our own prior study included both the amount of virus used for the immunizations and the method used for inactivation. In our follow study, rather than utilize the limiting amounts of autologous virus we could obtain directly from patient plasma [26], we developed procedures to optimize viral production by primary cell culture in order to increase the amount of autologous inactivated HIV-1 available for pulsing the autologous MDDM used at each vaccination.

The production of autologous inactivated HIV-1 to be used as immunogen in autologous therapeutic vaccines in humans entails significantly increased procedural complexity compared to the standard methods used to isolate and inactivate HIV-1 for basic laboratory research. Requirements include the use of: (i) cell culture methods that permit isolation and high level production of primary HIV isolates, in which standard reagents must be replaced by materials compatible with clinical grade GMP conditions, (ii) an inactivation procedure that provides the necessary level of safety with retention of antigenic integrity, (iii) a suitable method for concentration and purification of inactivated virions, (iv) suitable procedures to exclude the presence of adventitious agents, and finally (v) validation of a reproducible immunogen manufacturing process capable of meeting protocol defined product release specifications. All these steps and procedures must be in agreement with relevant regulatory guidelines, which are intended to minimize the risk of adverse effects [27,28].

We describe the detailed methodology for preparation and characterization of autologous whole inactivated HIV-1 for use in a pilot clinical trial to assess this immunization approach (Clinical Trial DCV-2, NCT00402142; Phase II Study of Autologous Myeloid Dendritic Cells as a "Cellular Adjuvant" for a Therapeutic HIV-1 Vaccine in Early Stage HIV-1+ Patients).

2. Material and methods

2.1. Patients

Sixty chronically HIV-1-infected patients were included in the Clinical Trial DCV-2 (NCT00402142); a randomized trial with placebo control and double blind masking to assess the safety and activity of a therapeutic HIV vaccine consisting of autologous myeloid dendritic cells pulsed ex vivo with high doses of inactivated autologous HIV-1 in infected patients in a very early stages of the disease (CD4 > 450×10^6/L) and plasma viral load (pVL) before any antiretroviral therapy greater than 10,000 HIV-1 RNA copies/mL. The trial is being performed in two parts: Part I involves patients off cART for a minimum period of 2 years ($n = 24$ patients: 12 cases and 12 placebo control); Part II involves patients on cART with pVL lower than 50 HIV-1 RNA copies/mL for a minimum period of six months ($n = 36$ patients: 24 cases and 12 placebo control). HIV-1 was isolated from the cases. A brief treatment interruption was required for the participants in Part II to allow virus isolation. The study was explained in detail to all patients, and all signed informed written consent. The clinical trial was approved by both the institutional Committee of Ethics and Clinical Investigation and the Spanish Agency of Drug and Health Products.

2.2. Culture medium and reagents

All reagents used to manufacture the heat inactivated autologous HIV-1 immunogen as a final product were sterile, endotoxin free and, either were themselves pharmaceutical products or were manufactured under GMP conditions. The manipulation of all opened tubes and reagents was always done under sterile conditions in bio II/A biosafety cabinets following the corresponding standard operational procedures (SOPs) validated during the preclinical evaluation of this project.

Cell cultures were established in T75 flasks (Corning Inc. 430641) using X-VIVO 20 media (Serum-free medium, Lonza-Biowhitaker) supplemented with 10% of AB human serum (ABHuS). Buffy coats and AB human plasma from anonymous numbered healthy HIV-negative blood bank donors were obtained (Banc de Sang i Teixits, Barcelona, Spain). They were tested negative for HBsAg, anti-HCV, anti-HIV-1+2, HCV-RNA, HIV-1-RNA, HBV-DNA and syphilis (TPHA) serology. Plasmas were converted into ABHuS by treatment with 10 U of human thrombin (Tissucol Duo®, Baxter) per mL of AB plasma, for 30 min at 37 °C, and centrifuged at $6000 \times g$ for 30 min. Afterwards, ABHuS was heat-inactivated at 56 °C for 30 min, centrifuged at $6000 \times g$ for 30 min and stored in aliquots at −20 °C until use. CD8+ lymphocytes were depleted from peripheral blood mononuclear cells (PBMCs) using CliniMACS Starting Kits, CD8 MicroBeads, (Miltenyi Biotec, Inc.) and sterile LS columns (Miltenyi Biotec, Inc.), which were certified endotoxin free (LAL Lonza). CD8 MicroBeads 7.5 mL vials were aliquoted into 0.1 mL sterile vials and frozen at −20 °C until usage before the manufacturer's expiry date. Pharmaceutical anti-CD3 (Orthoclone OKT3®) was prepared as a stock solution at a concentration of 10 μg/mL in X-