Vaccine 28 (2010) 5377–5385

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Evaluation of the immune response and protective effects of rhesus macaques vaccinated with biodegradable nanoparticles carrying gp120 of human immunodeficiency virus

Ai Himeno[a], Takami Akagi[b,d], Tomofumi Uto[c,d], Xin Wang[c,d], Masanori Baba[c,d], Kentaro Ibuki[a], Megumi Matsuyama[a], Mariko Horiike[a], Tatsuhiko Igarashi[a], Tomoyuki Miura[a,d,*], Mitsuru Akashi[b,d,**]

[a] Laboratory of Primate Model, Experimental Research Center for Infectious Diseases, Institute for Virus Research, Kyoto University, 53 Shogoinkawaramachi, Sakyo-ku, Kyoto 606-8507, Japan
[b] Department of Applied Chemistry, Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan
[c] Division of Antiviral Chemotherapy, Center for Chronic Viral Diseases, Graduate School of Medical and Dental Sciences, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima 890-8544, Japan
[d] Japan Science and Technology Agency (JST), Core Research for Evolutional Science and Technology (CREST), Saitama 332-0012, Japan

ARTICLE INFO

Article history:
Received 26 January 2010
Received in revised form 6 April 2010
Accepted 15 April 2010
Available online 14 May 2010

Keywords:
HIV vaccine
Biodegradable nanoparticles
Adjuvant

ABSTRACT

We previously reported that biodegradable amphiphilic poly(γ-glutamic acid) nanoparticles (NPs) carrying the recombinant gp120 env protein of the human immunodeficiency virus type 1 (HIV-1) were efficiently taken up by dendritic cells, and induced strong $CD8^+$ T cell responses against the gp120 in mice. To evaluate gp120-carrying NPs (gp120-NPs) as a vaccine candidate for HIV-1 infection, we vaccinated rhesus macaques with these gp120-NPs and examined the immune response and protective efficacy against a challenge inoculation of simian and human immunodeficiency chimeric virus (SHIV). We found that gp120-NP vaccination induced stronger responses for both gp120-specific cellular and humoral immunity than gp120-alone vaccination. After the challenge inoculation with SHIV, however, the peak value of viral RNA in the peripheral blood was higher in the vaccinated groups, especially the gp120-NP vaccinated group, than naive control group. Higher value of viral load was also maintained in gp120-NP vaccinated group. Furthermore, $CD4^+$ T cells from the peripheral blood decreased more in the vaccinated groups than the control group. Thus, induced immune responses against gp120 enclosed in NPs were not effective for protection but, conversely enhanced the infection, although the gp120-NPs showed a stronger induction of immune responses against the vaccinated antigen in rhesus macaques. These results support the importance of determining immune correlate of protective immunity for vaccine development against HIV-1 infection.

1. Introduction

The development of a human immunodeficiency virus type 1 (HIV-1) vaccine is much needed to prevent the continuing spread of the acquired immunodeficiency syndrome (AIDS) pandemic across the world [1]. The use of highly active antiretroviral therapy (HAART) has achieved a reduced death rate due to AIDS. HAART can efficiently suppress virus replication in HIV-1-infected individuals [2]. However, HAART is expensive, and the complete eradication of the virus from infected patients by HAART does not seem possible, suggesting the necessity for long-term treatment. Moreover, the side effects and emergence of drug resistant viruses limit the long-term application of HAART [3]. Thus, an effective, safe and affordable HIV-1 vaccine with prophylactic/therapeutic effects is the most desirable for the eradication of HIV-1 infection.

Vaccination to induce an adaptive immune response is expected for a broad range of infectious diseases. Traditional vaccines are mainly composed of live attenuated viruses or whole inactivated pathogens, and these vaccines often cause many unwanted side effects [4]. With recent advances in biotechnology, new vaccine strategies have been developed using part of the pathogen, such as recombinant/synthetic proteins or peptides, or DNA encoding for these proteins. Subunit vaccines are generally very safe, with well-defined components. However, these antigens are often

* Corresponding author at: Laboratory of Primate Model, Experimental Research Center for Infectious Diseases, Institute for Virus Research, Kyoto University, 53 Shogoinkawaramachi, Sakyo-ku, Kyoto 606-8507, Japan. Tel.: +81 75 751 3984; fax: +81 75 761 9335.
** Corresponding author at: Department of Applied Chemistry, Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita 565-0871, Japan. Tel.: +81 6 6879 7356; fax: +81 6 6879 7359.
E-mail addresses: tmiura@virus.kyoto-u.ac.jp (T. Miura), akashi@chem.eng.osaka-u.ac.jp (M. Akashi).

0264-410X/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.vaccine.2010.04.110

poorly immunogenic, and thus require the use of adjuvants or vaccine delivery systems to induce adequate immunity [5–7]. Particulate adjuvants (e.g. micro/nanoparticles, emulsions, ISCOMS, liposomes, virosomes and virus-like particles) have been widely investigated in HIV vaccine delivery systems [8]. Antigen uptake by antigen presenting cells (APCs) is enhanced by the association of these antigens with nano-sized particles. The adjuvant effect of the nanoparticles appears to be largely a consequence of their uptake into the APCs. Dendritic cells (DCs) are highly specialized APCs that can activate naive T cells, and hence initiate primary immune responses. Therefore, the active delivery of antigens to DCs is an important factor for the development of effective vaccines [9,10].

Vaccines to prevent HIV infection have focused on the induction of virus-specific neutralizing antibodies and cytotoxic T lymphocyte (CTL) responses. An important role of neutralizing antibodies for HIV-1 *env* has been demonstrated by the passive transfer of these neutralizing antibodies in animal models. The passive transfer of various human monoclonal antibodies can protect against viral challenge [11–13]. However, it should be noted that for the protection of viral transmission, a high-titer and an enormous quantity of antibodies are needed. Similarly, HIV-1-specific CTL responses have also been associated with the control of HIV-1 infection. The importance of CTL for HIV-1 infection is suggested by the inverse correlation between anti-HIV-1 CTL responses and the virus load in humans [14,15]. In addition, the depletion of $CD8^+$ T cells through the infusion of anti-CD8 antibodies decreases the control of viremia in infected macaques [16,17]. Therefore, recent vaccine approaches have focused on eliciting CTL responses [18]. To solve the problem of the poor immunogenicity of HIV-1 *env*, several candidate adjuvants and delivery systems are currently being investigated in rhesus macaques [19–22]. In fact, the first phase III trial performed using the HIV-1 gp120-based vaccine candidate AIDSVAX from VaxGen was a failure [23]. Varying degrees of protection have been demonstrated in a number of vaccine trials employing the use of a pathogenic simian immunodeficiency virus (SIV) or a chimeric simian/human immunodeficiency virus (SHIV) as the challenge virus [24].

In previous studies, we demonstrated that intranasal immunization with inactivated HIV- or SHIV-capturing polystyrene nanospheres (HIV-NS or SHIV-NS) could induce vaginal anti-HIV-1 gp120 IgA and IgG antibodies in mice [25–27] and macaques, and that SHIV-NS-immunized macaques exhibited partial protection when vaginally and systemically challenged with pathogenic viruses [28]. These results clearly indicated that HIV-1-capturing nanospheres are useful as adjuvant carriers for a prophylactic vaccine against HIV-1 infection. However, both biodegradability and biocompatibility of the adjuvant carriers are required for medical use. Therefore, the development of biodegradable nanoparticles is indispensable for clinical applications [29]. To that end, we have recently prepared protein-loaded biodegradable nanoparticles composed of hydrophobically modified poly(γ-glutamic acid) (γ-PGA) [30–33]. γ-PGA is a naturally occurring water-soluble, biodegradable, edible and non-toxic poly(amino acid)s that is synthesized by certain strains of *Bacillus*. γ-PGA hydrophobic derivatives (γ-hPGA) formed 200 nm-sized nanoparticles (NPs) in water. These protein-encapsulated γ-hPGA NPs were efficiently taken up by immature mouse DCs. These γ-hPGA NPs also had adjuvant activity for DC maturation. Thus, γ-hPGA NPs have significant potential as an antigen carrier and as an adjuvant for DCs [34,35]. Moreover, immunization with HIV-1 gp120- or p24-encapsulated γ-hPGA NPs strongly induced antigen-specific cellular immunity in mice [35–37]. These results suggest that HIV-1-related antigen-carrying γ-hPGA NPs provide a novel delivery system, and function as an adjuvant for vaccination against HIV-1 infection.

In this study, we evaluated the immune responses in macaques after intranasal and subcutaneous immunization with HIV-1 gp120-carrying γ-hPGA NPs (gp120-NPs). Moreover, to determine whether the vaccination by gp120-NPs can enhance the protective effect against pathogenic viruses, the macaques were intravenously challenged with SHIV-KU-2. Here we report the use of nanoparticles as HIV-1 vaccine adjuvants. Our results demonstrated that gp120-NPs have great potential for the induction of HIV-1 gp120-specific cellular and humoral immunity. However, the macaques immunized with gp120-NPs had an augmented viral load. These results may be helpful for the design of HIV-1 vaccines.

2. Materials and methods

2.1. Nanoparticles (NPs)

γ-PGA (number-average molecular weight, $M_n = 3.8 \times 10^5$) was kindly provided by Meiji Seika Co., Ltd., Tokyo, Japan. The synthetic procedures for the γ-hPGA NPs consisting of γ-PGA conjugated with L-phenylalanine ethylester (γ-PGA-*graft*-Phe) and protein-carrying γ-hPGA NPs have been described previously [35,36]. The mean diameter of the γ-hPGA NPs in aqueous solution was measured by a dynamic light scattering (DLS) method using a Zetasizer Nano ZS (Malvern Instruments, UK). The diameter of the NPs was about 200 nm.

2.2. Preparation of gp120-encapsulated γ-hPGA NPs for intranasal vaccination

Recombinant HIV-1 III_B envelope glycoprotein gp120 (Immuno Diagnostics, Woburn, MA) was chosen for the immunization experiments, and encapsulated into the γ-hPGA NPs (gp120-NPs). To prepare the gp120-encapsulated γ-hPGA NP, γ-PGA-*graft*-Phe (10 mg/ml in DMSO) was added to the same volume (500 μl) of 500 μg/ml recombinant gp120 to yield a translucent solution. The resulting solution was centrifuged at 14,000 × *g* for 15 min, repeatedly rinsed to remove the organic solvents, and prepared to a final particle concentration of 20 mg/ml. The gp120 loading content into the NPs was measured by the Lowry method, as previously described [35]. The amount of encapsulated gp120 into the NPs was 10 μg per mg NP.

2.3. Preparation of gp120-surface immobilized γ-hPGA NPs for subcutaneous vaccination

To prepare the gp120-immobilized γ-hPGA NPs, the carboxyl group of the γ-hPGA NPs (10 mg/ml) was first activated by water-soluble carbodiimide (1 mg/ml) for 20 min. The NPs (5 mg/ml) obtained by centrifugation (14,000 × *g* for 15 min) were suspended in 125 μg/ml gp120, and the mixture was incubated at 4 °C for 24 h. After the reaction, the centrifuged NPs were washed twice with PBS. The resulting solution was prepared to a final particle concentration of 20 mg/ml. The amount of gp120 immobilized onto the NPs was 10 μg per mg NP.

2.4. Animals

Nine rhesus macaques (*Macaca mulatta*) of the Indian origin, which spread in Japan, were used in this study. All macaques were serologically negative for simian immunodeficiency virus (SIV) and simian T cell lymphotropic virus type 1. The macaques were housed in P3 level isolators throughout the experimental period. All experiments were carried out in accordance with regulations approved by the Institutional Animal Care and Use Committee of the Institute for virus Research, Kyoto University.