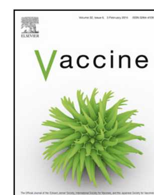
Vaccine 32 (2014) 2501–2506

Contents lists available at ScienceDirect

Vaccine

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



Exploring mucosal immunization with a recombinant influenza virus carrying an HIV-polyepitope in mice with pre-existing immunity to influenza

Bruno Garulli [a,b], Giuseppina Di Mario [a], Maria Giuseppina Stillitano [a], Yoshihiro Kawaoka [c,d,e], Maria Rita Castrucci [a,*]

[a] Department of Infectious, Parasitic and Immune-Mediated Diseases, Istituto Superiore di Sanità, 00161 Rome, Italy
[b] Department of Biology and Biotechnology "Charles Darwin", Sapienza University of Rome 00185 Rome, Italy
[c] Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, USA
[d] Division of Virology, Department of Microbiology and Immunology, and International Research Center for Infectious Diseases, Institute of Medical Science, University of Tokyo, Japan
[e] Infection-Induced Host Responses Project, Exploratory Research for Advanced Technology, Saitama 332-0012, Japan

ARTICLE INFO

Article history:
Received 18 October 2013
Received in revised form 7 February 2014
Accepted 25 February 2014
Available online 21 March 2014

Keywords:
AIDS vaccine
Viral vector
CD8+ T cell responses
Pre-existing antibody
Mucosal immunity

ABSTRACT

HIV-1 vaccines based on recombinant vectors have been developed to elicit immune responses; however, the failure of the STEP HIV-1 vaccine trial has caused concern regarding the impact on vaccine efficacy of pre-existing vector seropositivity in humans. By using a mouse model of infection, we evaluated the immune responses elicited by intranasal and vaginal immunization with the recombinant influenza virus WSN/CKG carrying the PCLUS3-P18 peptide and a Gag epitope in its hemagglutinin, and the impact of pre-existing vector immunity on protection against recombinant vaccinia virus challenge. We found that despite the protective immunity induced in naïve mice by the WSN/CKG virus via either route, the vaginal immunization of mice with pre-existing influenza immunity restricted vPE16 replication more significantly in the ovaries than intranasal immunization. Thus, successful vaccination strategies under limiting conditions, such as pre-existing vector immunity, require the local induction of mucosal immunity at the site of virus infection.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

A successful HIV vaccine still represents a promising strategy for the prevention of AIDS [1,2]. Various vaccine vectors expressing different HIV-1 antigens have been assayed to determine the magnitude of the immune response, and the correlation with vaccine-induced protection against challenge in animal models [3,4]. Among them, recombinant adenovirus vectors, used for this purpose in clinical trials, have raised some concerns regarding reduced immunogenicity and vaccine failure that appears to correlate with pre-existing vector-specific immunity [5]. This has led to a better understand as to what extent pre-existing immunity against vaccine vectors could influence the immunogenicity of the vector-delivered HIV-1 antigens [6–8].

Recombinant influenza viruses engineered to express foreign antigens have been shown to induce effective immune responses in mice [9,10]. Their ability to infect and induce full maturation of dendritic cells is critical for effective antigen-presentation and induction of immunity [11,12]. Influenza viruses continue to cause epidemics in human populations, and the cross-reactive immune responses to the conserved influenza proteins detectable in healthy adults might be responsible for the poor efficacy of this virus as a vector of foreign antigens in humans [13,14]. Indeed, in a recent study performed in mice, pre-existing immunity to influenza was shown to considerably compromise the immunogenicity of a foreign antigen carried by a recombinant influenza virus [15]. We previously found that vaginal (i.vag.) immunization of progesterone-treated female mice with a recombinant influenza virus bearing an HIV CTL epitope in the neuraminidase (NA) stalk of A/WSN/33 (H1N1) (WSN) virus induced a vigorous and durable HIV-1-specific CD8+ T cell response in both mucosal and systemic compartments [16]. Thus, this mouse model of infection and the use of recombinant influenza viruses bearing foreign antigens could be useful to dissect further the influence of anti-vector immunity to heterologous influenza viruses on the development of antigen-specific immune responses.

* Corresponding author. Tel.: +39 06 49903259; fax: +39 06 49902082.
E-mail address: mariarita.castrucci@iss.it (M.R. Castrucci).

http://dx.doi.org/10.1016/j.vaccine.2014.02.077
0264-410X/© 2014 Elsevier Ltd. All rights reserved.

Here, we examined the characteristics of the immune responses elicited by infection of the respiratory or genital tracts of mice with a recombinant influenza virus (WSN/CKG) bearing a synthetic HIV polyepitope in its hemagglutinin (HA), and the extent of protection against viral challenge with recombinant vaccinia viruses in the ovarian tissue of mice with prior immunity to an heterologous influenza virus with antigenically distinct surface proteins but conserved internal antigens.

2. Materials and methods

2.1. Generation of recombinant WSN/CKG virus

The synthetic DNA construct (named CKG) containing the coding sequences for the cluster peptide PCLUS3 and the peptide 18 (P18) from the envelope glycoprotein gp160 of the HIV-1 III isolate [17,18], and the HIV-1 Gag-residues 192–208, containing the CTL epitope Gag_{197} [19], was introduced into the cloning cassette previously generated at the 3′ end of the signal peptide coding sequence of the pPolI–WSN HA plasmid [20].

The recombinant WSN/CKG (H1N1) virus bearing the CKG polyepitope at the N terminus of the HA protein was generated by using plasmid-based reverse genetics [21]. WSN/CKG virus was growing in MDCK cells with average titers of 6.3–7 log plaque-forming units (PFU)/ml. Stable expression of the 67 amino acid-long insert was confirmed by direct sequencing of RT-PCR products derived from plaques after several passages of growth in MDCK cells. RT-PCR amplification and sequence reactions were performed using primers for the HA gene [5′-GGCAAAACTACTGGTCCTGT-3′ (forward), 5′-TACTGAGCTCAATTGCTCCC-3′ (reverse)].

2.2. Immunization of mice

All animal work was performed in compliance with institutional guidelines and approved protocols. Female BALB/c mice were anesthetized with Avertin before being intranasally (i.n.) infected with 45 μl of PBS containing 10^6 PFU of WSN/CKG virus. For i.vag. infection, groups of mice were subcutaneously injected with 3 mg of progesterone (Pharmacia & Upjohn) [16]. Five days later, they were i.vag. infected with 10^6 PFU of WSN/CKG virus in a 10 μl volume.

For experiments involving animals with pre-existing immunity to influenza, mice were i.n. infected six weeks earlier with 10^5 PFU of the H3N2 reassortant virus X31 (A/Aichi/2/1968 (H3N2) HA and NA and A/PR/8/34 (H1N1) internal genes), and then immunized either i.n. or i.vag. with 10^6 PFU of WSN/CKG virus.

2.3. Viral replication in murine respiratory and genital tracts

To determine viral spread and replication in mouse respiratory tract, mice were anesthetized and infected i.n. with 10^6 PFU of WSN/CKG virus, or with 10^3 PFU of WSN virus. Four days after infection, mice were sacrificed, and virus titers were determined in lung homogenates and nasal turbinates by titration in MDCK cells.

Vaginal lavages were obtained at various times post-infection (p.i.) by rinsing the vaginal cavity with sterile PBS, and virus titers were determined by titration in MDCK cells.

2.4. IFN-γ-specific ELISPOT assay

Spleens and lymph nodes draining the respiratory tracts (mediastinal lymph nodes, MLN) and the vaginal tracts (iliac lymph nodes, ILN) of immunized mice were collected at the indicated time points p.i. and assayed for antigen-specific IFN-γ-producing cells by using an ELISPOT assay. A single-cell suspension from lymphoid tissues was cultured with the indicated synthetic peptides in anti-IFN-γ-coated plates at 37 °C for 36 h. Colored spots representing IFN-γ-releasing cells are reported as the number of spot-forming cells (SFC) per 10^6 cells.

2.5. ELISA

The presence of P18IIIB-specific IgG antibodies in serum from mice immunized with WSN or WSN/CKG virus was determined by means of an ELISA (18). Briefly, 96-well plates were coated overnight with 10 μM P18 peptide in 0.1 M carbonate buffer pH 9.6. Plates were blocked with 1% BSA in PBS, and serial two-fold dilutions of serum samples in PBS were added to the wells. After a 2-h incubation, plates were washed with PBS containing 0.05% Tween 20, and incubated with an HRP-goat anti-mouse IgG antibody. Bound antibodies were detected by the addition of TMB (Vector), and absorbance was read at a wavelength of 450 nm.

2.6. Protection against challenge infection with vaccinia viruses

The virus challenge experiments were performed by using recombinant vaccinia viruses expressing the full-length HIV-1IIIB gp160 (vPE16), and the HIV-1IIIB Gag gene (vDK1) [22,23]. Vaccinated mice were intraperitoneally (i.p.) challenged with 10^6 PFU of vaccinia viruses diluted in 300 μl of PBS. Six days after the virus challenge, ovaries from individual mice were homogenized in MEM/2% FCS and lysed by repeated freeze/thaw cycles. Titers were determined on infected CV-1 cells after 48 h by staining with 0.1% crystal violet. The limit of viral detection was 2 $\log_{10}$ PFU/ml. Statistical significance was determined by using the Student's t-test.

3. Results

3.1. Generation of a chimeric influenza virus expressing the CKG polyepitope and its replication in mice

A peptide construct containing the multideterminant Th peptide PCLUS3 from the gp160 of HIV-1, and P18 of the V3 loop, corresponding to the principal neutralizing determinant of HIV-IIIB and to the major immunodominant cytotoxic T cell epitope (P18–I10) in mice, as well as being recognized by human CTLs, was previously described [17,18]. Several studies have examined this HIV-1 peptide vaccine in mice [24,25]. Therefore, the CKG construct containing the PCLUS3–P18 domain-coding sequences, together with the CTL epitope Gag_{197} of HIV-1, were inserted into the HA of WSN virus, and the WSN/CKG virus was generated and used in our studies (Table 1).

The growth kinetics of the WSN/CKG virus in MDCK cells were slightly reduced compared with those of WSN virus (Fig. 1A). To evaluate the replication of the WSN/CKG virus in vivo, mice were inoculated i.n. with the recombinant virus and its growth kinetics were compared with that of the parental WSN virus. The viral loads in the nasal turbinates and lungs measured on day 4 p.i. in mice infected i.n. with 10^6 PFU of WSN/CKG virus were about two logs lower than those observed for mice infected with 10^3 PFU of WSN virus (Fig. 1B). Furthermore, viral replication in the vaginal mucosa was evident with the highest titers of WSN virus for up to day 6 p.i. being measured in the vaginal washes of progesterone-treated mice that had been infected via the i.vag. route, whereas WSN/CKG virus replicated less efficiently than did WSN virus and was completely cleared by day 5, even though a higher virus inoculum was used (Fig. 1C). Given that WSN/CKG virus was highly attenuated in vivo, an inoculum containing 10^6 PFU of virus was subsequently used for all experiments involving immunizing mice.

Download English Version:

https://daneshyari.com/en/article/10965173

Download Persian Version:

https://daneshyari.com/article/10965173

Daneshyari.com