Trials in Vaccinology 3 (2014) 95–101

Contents lists available at ScienceDirect

Trials in Vaccinology

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

Bupivacaine enhances the magnitude and longevity of HIV-specific immune response after immunization with a CD4 epitope-based DNA vaccine

Susan Pereira Ribeiro [a,d,1], Juliana de Souza Apostólico [a,b,1], Rafael Ribeiro Almeida [a,d], Jorge Kalil [a,c,d], Edecio Cunha-Neto [a,c,d], Daniela Santoro Rosa [a,b,d,*]

[a] Laboratory of Clinical Immunology and Allergy-LIM60, University of São Paulo-School of Medicine, Brazil
[b] Division of Immunology, Federal University of São Paulo (UNIFESP), Brazil
[c] Heart Institute (InCor), University of São Paulo-School of Medicine, São Paulo, Brazil
[d] Institute for Investigation in Immunology-INCT, São Paulo, Brazil

ARTICLE INFO

Article history:
Received 20 November 2013
Revised 6 May 2014
Accepted 13 May 2014

Keywords:
Bupivacaine
CD4 epitopes
HIV
DNA vaccine

ABSTRACT

The development of an effective HIV vaccine is still a major scientific challenge. HIV vaccine trials conducted until now were not able to induce broad neutralizing antibodies or effective cell mediated immune responses. More recently, $CD4^+$ T cells have been shown to play an important role in viral control and better disease prognosis. We have recently developed a DNA vaccine encoding 18 conserved multiple HLA-DR-binding HIV-1 CD4 epitopes (HIVBr18), capable of eliciting broad $CD4^+$ T cell responses in BALB/c and in multiple HLA class II transgenic mice. Despite the advantages of DNA vaccines and a large number of clinical trials, it has been a challenge to transfer the success of inducing potent immunity observed in animal models to humans. Here, we sought to evaluate the potential use of bupivacaine, a local anesthetic, as an adjuvant for HIVBr18. We observed that the concomitant administration of the local anesthetic bupivacaine with the DNA vaccine HIVBr18 increased the magnitude of $CD4^+$ and $CD8^+$ T cell responses and cytokine production without compromising their breadth. Furthermore, we demonstrate that coadministration of bupivacaine also impacted the longevity of specific immune responses. Since bupivacaine is used in clinical settings, we believe that this concept may contribute to overcome the limited immunogenicity of DNA vaccines in humans.

Introduction

Human immunodeficiency virus type 1 (HIV-1) infection and the incurable disease it causes, the acquired immunodeficiency syndrome (AIDS), remains a major public health problem in endemic countries. Despite the successful development of new antiretroviral drugs in the last years, there is still no vaccine available.

In recent years, several studies have aimed to develop new strategies for an effective vaccine to prevent HIV infection. It is widely accepted that an effective prophylactic vaccine against HIV-1 should elicit neutralizing antibodies (Nabs) that, if present at the time of transmission, could block HIV acquisition. However, all vaccine candidates based on the induction of neutralizing antibodies failed to induce Nabs and also failed large-scale efficacy phase III trials [1,2]. Therefore, the HIV prophylactic vaccine research shifted to evaluate vaccine candidates capable of inducing cell-mediated immune responses (CMI). A vaccine that elicits CMI will be able to limit viral transmission and prevent HIV-1 associated disease progression by controlling viral loads in those individuals who become infected [3–5].

Although five vaccine candidates were tested in phase IIb/III efficacy trials, only one demonstrated some level of protection [6]. The recent phase III clinical trial RV144 was the first to demonstrate a modest evidence of protection against acquisition of HIV-1 infection among vaccines in the absence of serum-neutralizing antibodies, with an estimated vaccine efficacy of 31.2% [7,8]. Preliminary analysis of the protective immune responses revealed that most vaccinated HIV-negative individuals presented predominantly polyfunctional effector $CD4^+$ T cell responses against the V2 region of the envelope protein [9].

Control of viral infections is crucially dependent on $CD4^+$ T cell responses [10]. In this scenario, $CD4^+$ T cells are mainly implicated

* Corresponding author at: Laboratory of Experimental Vaccines, Division of Immunology, Federal University of São Paulo (UNIFESP), Rua Botucatu, 862, 4° andar, 04023-062 São Paulo, SP, Brazil. Tel.: +55 1155764848.
E-mail address: dsantororosa@gmail.com (D.S. Rosa).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.trivac.2014.05.001
1879-4378/

in helping $CD8^+$ cytotoxic T cell responses (CTL), as well as in supporting B cells through antibody class switch recombination and affinity maturation. During HIV infection, and also in simian immunodeficiency virus (SIV) infection in nonhuman primates, $CD4^+$ T cells can suppress virus replication directly (through the release of perforin and granzymes) or indirectly (through the release of soluble antiviral chemokines), maintain mucosal immunity by producing IL-17, and produce cytokines to help B and $CD8^+$ T cells (for a review see [11]). In SIV infection, specific $CD4^+$ T cells can contribute directly to effective suppression of the virus by inhibiting SIV replication in macrophages [12]. More recently, it was demonstrated that HIV-infected individuals with lower set point viral load, slower disease progression and better clinical outcome in the absence of antiretroviral therapy, display significant expansion of HIV-specific $CD4^+$ T cell responses. Moreover, these subjects present enhanced $CD4^+$ T cell cytolytic activity and higher frequency of IFN-γ producing cells [13]. It is thus clear that a successful HIV vaccine should also induce strong $CD4^+$ T cell responses [14].

We have identified a group of conserved CD4 epitopes from HIV that were able to bind to multiple HLA-DR molecules. Synthetic peptides representing such epitopes were recognized by PBMC from over 90% of HIV-1 infected individuals [15]. More recently, we reported that a DNA vaccine and a recombinant adenovirus encoding such epitopes (HIVBr18) were able to induce broad specific $CD4^+$ and $CD8^+$ T cell responses in BALB/c [16,17] and in transgenic mice to human HLA class II alleles (HLA-DR2, -DR4, -DQ6, -DQ8) [18]. Functional profile analysis induced by these vaccines demonstrated that HIVBr18 was able to induce high magnitude, broad and polyfunctional $CD4^+/CD8^+$ T cell responses, and 8 out of 18 vaccine-encoded peptides were recognized. Moreover, the vaccine also generated long-lived central and effector memory T cells [16].

Immunization using plasmid DNA is a promising technology for gene delivery. It offers several potential advantages over conventional approaches, including safety profile and feasible production method [19]. Despite the advantages and a large number of clinical trials, it has been a challenge to transfer the success of inducing potent immunity observed in animal models to humans. The mechanism of such phenomenon is not fully understood, but it is likely that inefficient transfection is a major determinant [20]. Such observations have led to the pursuit of alternative strategies to enhance immune responses to the encoding antigen [21]. One alternative to improve the immunogenicity of DNA vaccines is the concomitant use of adjuvants. Adjuvants (from the latin *adjuvare* = help) are substances that when incorporated into a vaccine formulation, enhance its immunogenicity. Addition of such adjuvants increases breadth, magnitude and also skews the type of the immune response [21]. They can also lead to a reduction of the dose and/or number of immunizations for a given vaccine [22–24] and even increase seroconversion rates in populations with reduced responsiveness [25,26]. To increase the immunogenicity of DNA immunization using HIV antigens, genes coding cytokines [27–30] and also toll like receptors (TLRs) agonists [31,32] have been used.

Chemical compounds have been also evaluated as adjuvants for DNA vaccines [33]. One of such compounds is bupivacaine or marcaine, a local anesthetic drug belonging to the *amino amide* group that blocks neuron transmission. Bupivacaine is a myotoxin that when injected destroys myofiber cells leading to the clearance of cell debris and proliferation of myoblasts [34]. In addition, the recruitment of inflammatory cells to the site of bupivacaine injection may allow for transfection of immune cells [35]. Pretreatment of muscle with 0.25–0.5% bupivacaine prior to DNA injection increases gene expression by 30–50-fold, resulting in an enhancement of the immune responses [34,36]. Also, the complex formed with bupivacaine protects DNA from nuclease degradation, and intramuscular immunization with this formulation results in higher immune responses against the encoded antigen [33,37]. Further in support of the adjuvant effect of bupivacaine, mucosal immunization with a DNA vaccine encoding an HIV-1 envelope protein was able to elicit vaginal immunoglobulins that specifically bound to the HIV-1 envelope and neutralized HIV-1 infectivity *in vitro* [38]. Recently, bupivacaine was used in combination with a DNA vaccine encoding *Streptococcus mutants* antigens, and intranasal or intramuscular immunization induced antibodies, IFN-γ production and significant reduction in dental caries lesions [39].

In the present study, we analyzed the adjuvant properties of the concomitant use of bupivacaine on the cellular immune response against the epitopes encoded by the DNA vaccine HIVBr18. Our data suggest that the administration of a DNA vaccine encoding HIV CD4 T cell epitopes together with bupivacaine at the time of immunization results in enhancement of the magnitude and longevity of antigen-specific cellular immune responses.

Material and methods

DNA vaccine

We previously designed a multiepitope construct containing the mammalian codon optimized nucleotide sequences of the 18 HIV-1 CD4 epitopes described previously [15]: p17 (73–89), p24 (33–45), p24 (131–150), p6 (32–46), pol (63–77), pol (136–150), pol (785–799), gp41 (261–276), gp160 (19–31), gp160 (174–185), gp160 (188–201), gp160 (481–498), rev (11–27), vpr (58–72), vpr (65–82), vif (144–158), vpu (6–20) and nef (180–194). The artificial gene (EZBiolab) was cloned into the pVAX1 vector (Invitrogen) to generate the HIVBr18 vaccine, as previously described [16,18]. The DNA vaccine was purified using the Endofree Plasmid Giga Kit from Qiagen according to manufacturer's instructions.

Mice and immunization

BALB/c mice were purchased from Centro de Desenvolvimento de Modelos Experimentais para Medicina e Biologia (CEDEME) and housed at the experimental animal facilities at the Division of Immunology, Federal University of São Paulo (UNIFESP) under specific pathogen-free conditions. In all experiments, groups of 6 mice were immunized intramuscularly (IM) three times, 2 weeks apart, with 100 μg of the DNA vaccine HIVBr18 in the presence or absence of 0.2% bupivacaine hydrochloride (Sigma). Control mice received 100 μg of the empty vector pVAX in the presence or absence of 0.25% bupivacaine hydrochloride. A volume of 50 μL was injected into each quadriceps.

Ethics statement

All experiments were performed in accordance to the guidelines of the Ethics committee of Federal University of São Paulo (UNIFESP) and approved under protocol number 0121/11.

Peptide synthesis

The eighteen multiple HLA-DR binding peptides derived from the conserved regions of HIV-1 B subtype consensus were synthesized by GL Biochem (Vancouver, Canada). Peptide purity and quality were assessed by reverse-phase high performance liquid chromatography and mass spectrometry and were routinely above 90%.

Spleen cells isolation

Two or twelve weeks after the last dose, mice were euthanized and the spleen was removed aseptically. After obtaining single cell suspensions, cells were washed in 10 mL of RPMI 1640. Cells were