Biochimie 142 (2017) 179–182

Contents lists available at ScienceDirect

Biochimie

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

Short communication

ClearColi BL21(DE3)-based expression of Zika virus antigens illustrates a rapid method of antibody production against emerging pathogens

Wildriss Viranaicken [a, *], Brice Nativel [b], Pascale Krejbich-Trotot [a], Wissal Harrabi [a], Sandra Bos [a], Chaker El Kalamouni [a], Marjolaine Roche [a], Gilles Gadea [a], Philippe Desprès [a]

[a] PIMIT, Processus Infectieux en Milieu Insulaire Tropical, Université de La Réunion, INSERM UMR 1187, CNRS 9192, IRD 249, Plateforme CYROI, 2, rue Maxime Rivière, 97490, Sainte-Clotilde, Ile de La Réunion, France
[b] DéTROI, Diabète athérothrombose Thérapies Réunion Océan Indien, Université de La Réunion, INSERM UMR 1188, Plateforme CYROI, 2, rue Maxime Rivière, 97490, Sainte-Clotilde, Ile de La Réunion, France

ARTICLE INFO

Article history:
Received 9 June 2017
Accepted 14 September 2017
Available online 20 September 2017

Keywords:
Polyclonal antibody
Recombinant protein
Zika antigens
Inclusion bodies
Wistar rat

ABSTRACT

Available rapid, simple and accurate methods for detection and diagnosis of emerging viral diseases are required. Recently, there was an urgent need for specific antibodies against mosquito-borne Zika virus (ZIKV), which is an emerging zoonotic disease of medical concern in different regions of the world. Here, we showed that overexpression of ZIKV antigens in ClearColi BL21(DE3), a bacteria strain expressing a non-endotoxic form of LPS, is suitable for the production of specific ZIKV antisera. Two major ZIKV antigenic domains, the domain III from envelope E glycoprotein, which brings the virus-specific epitopes, and the N-terminal region of nonstructural NS1 glycoprotein, which is responsible for pathophysiological conditions, were overexpressed in ClearColi BL21(DE3). Immunization of adult rat with insoluble recombinant ZIKV antigens in inclusion bodies resulted in the production of specific antibodies in a few weeks. Anti-E and anti-NS1 antibodies are efficient as biological tools for ZIKV detection by indirect ELISA and immunoblot assay. This method could successfully be applied to any emerging viruses.

1. Introduction

An availability in antibody is required for *in situ* detection of a newly identified pathogen as well as diagnosis of the pathogen-related disease by screening tests. Neglected or emerging viral diseases are often lacking high quality antibodies. It is usual that available pathogen antibodies show a mediocre specificity and/or sensitivity because the more relevant antigens are poorly characterized or display an unsatisfying immunogenicity in animals. Mosquito-transmitted Zika fever is considered as emerging infectious disease of medical concern worldwide [1]. Phylogenetically, Zika virus (ZIKV) is mainly classified into African and Asian genotypes, the latter being involved in the recent epidemics in South Pacific, Latin America and Caribbean islands [2]. ZIKV infection can cause severe neurological disorders and birth defects (microcephaly) in humans [3]. To face ZIKV emergence, it is urgent to dispose of efficient anti-Zika antibodies as immunological tools for the detection of ZIKV *in vitro* and *in vivo*. Thus, we decided to produce major viral ZIKV antigens in bacteria expression system in order to generate specific anti-ZIKV antibodies in rodent. Like other medically important related mosquito-borne RNA viruses such as dengue (DV), West Nile (WNV), yellow fever (YFV) and Japanese encephalitis, ZIKV contains a positive-stranded genomic RNA which is translated into a large and unique polyprotein, the latter being processed into structural proteins (C, prM/M and E), and nonstructural (NS) proteins, NS1 to NS5, by host and viral proteases [4]. Flavivirus E and NS1 proteins are two major antigenic markers for viral infection. The E protein mediates virus cell entry process (receptor binding and membrane fusion). It has three structural distinct domains: a central ß-barrel shaped domain I (EDI), a finger-like domain II (EDII) and a C-terminal immunoglobulin-like domain III (EDIII) [5]. Several reports have shown that flavivirus type-specific neutralizing antibodies are mainly linked to EDIII which is stabilized by a single disulfide bridge [6]. The flavivirus glycosylated NS1 protein associates with lipids to form a homodimer (46–55 kDa) inside the cells. The intracellular form of NS1 contributes to viral replication, whereas the extracellular NS1 exists as

* Corresponding author.
E-mail address: wildriss.viranaicken@univ-reunion.fr (W. Viranaicken).

https://doi.org/10.1016/j.biochi.2017.09.011
0300-9084/

a hexameric lipoprotein, which interacts with the host factors leading to pathophysiological conditions [7].

A crucial step in the production of viral antigen antibody is the expression and purification of an immunogenic protein that is suitable for the stimulation of humoral response in animal recipient. It is common that overexpression of recombinant viral antigens in *E. coli* leads to inclusion body formation that can be isolated from crude cell lysates by action of detergents or chaotropic agents [8]. Because the protein refolding is frequently unsuitable, the recovered recombinant antigens are often deficient in their biological properties or antigenicity. As alternative to step purification of inclusion bodies, the crude cell lysate can be directly inoculated in animals but such method often causes acute inflammatory response to endotoxins, more frequently lipopolysaccharides (LPS), and then lethality [9]. In the present study, we showed that immunization of adult rats with non-purified recombinant ZIKV antigens overexpressed in ClearColi BL21(DE3), a bacteria strain expressing a non-endotoxic form of LPS, stimulates the production of specific antibodies. The efficient expression of recombinant antigenic domains from E (EDIII) and NS1 (the N-terminal region) as viral immunogens has allowed the rapid and efficient production of monospecific immune sera that are useful biological tools for ZIKV detection.

2. Results and discussion

2.1. ZIKV antigens are express as inclusion bodies in E. coli

In order to produce anti-ZIKV antibody, EDIII and the N-terminal region of NS1 (NS1-1 to NS1-151) from Asian ZIKV clinical isolate FP-25013-18 [1] were selected for viral antigen production in *E. coli*. The EDIII contains ZIKV-specific epitopes which have been reported to stimulate the production of anti-ZIKV neutralizing antibody [6]. It is of interest to note that antigenic cross-reactivity has been observed between ZIKV and other medically important flaviviruses such as DV, WNV, and YFV mainly due to ED I/II epitopes [10]. Mounting evidence indicts that ZIKV NS1 antibodies are largely virus-specific compare to E protein [11,12]. It has been reported that the N-terminal region of NS1 is sufficient to stimulate the production of anti-NS1 antibody [13,14].

To produce recombinant EDIII (rEDIII) and fragment $NS1^{1-151}$ in *E. coli*, synthetic genes with optimized codons to improve protein expression in bacteria cells were cloned into pET28a plasmid. An in-frame polyhistidine-tag was added to the C-terminus of viral antigens. The sequences of ZIKV rEDIII and $rNS1^{1-151}$ are listed in supplementary data. The corresponding recombinant plasmids pET28/ZIKV.rEDIII and pT28/ZIKV.$rNS1^{1-151}$ were transformed into ClearColi BL21 (DE3) bacteria, which contain a genetically modified LPS that does not trigger an endotoxic response [15]. The bacterial clones obtained were expanded and induced with 1 mM IPTG at 37 °C during 4 h in order to test the expression of the recombinant viral antigens. Bacterial cultures were lyzed by sonication and centrifuged. The collected protein fractions (total extracts, supernatants and pellets) were analyzed by SDS-PAGE (Fig. 1A). Coomassie Blue staining showed that rEDIII (apparent molecular weight 12 kDa) and $rNS1^{1-151}$ (apparent molecular weight 20 kDa) were essentially recovered as insoluble aggregates, which correspond to inclusion bodies. To circumvent this issue, we tested different strategies. We were not able to obtain soluble rEDIII and $rNS1^{1-151}$ using bacterial strains engineered to favor refolding like Origami2 or shuffleT7. Refolding rates were not either increased by the addition in the culture media of chemical agents like sorbitol, glycerol, high salt, betaine, sucrose or $MgCl_2$ [8,16] (data not shown). The fusion of DV antigens to thioredoxin has previously been reported to increase recombinant protein solubility [6,17,18]. In our hands, thioredoxin fusion showed no effect on the insolubility of ZIKV rEDIII and $rNS1^{1-151}$ (data not shown).

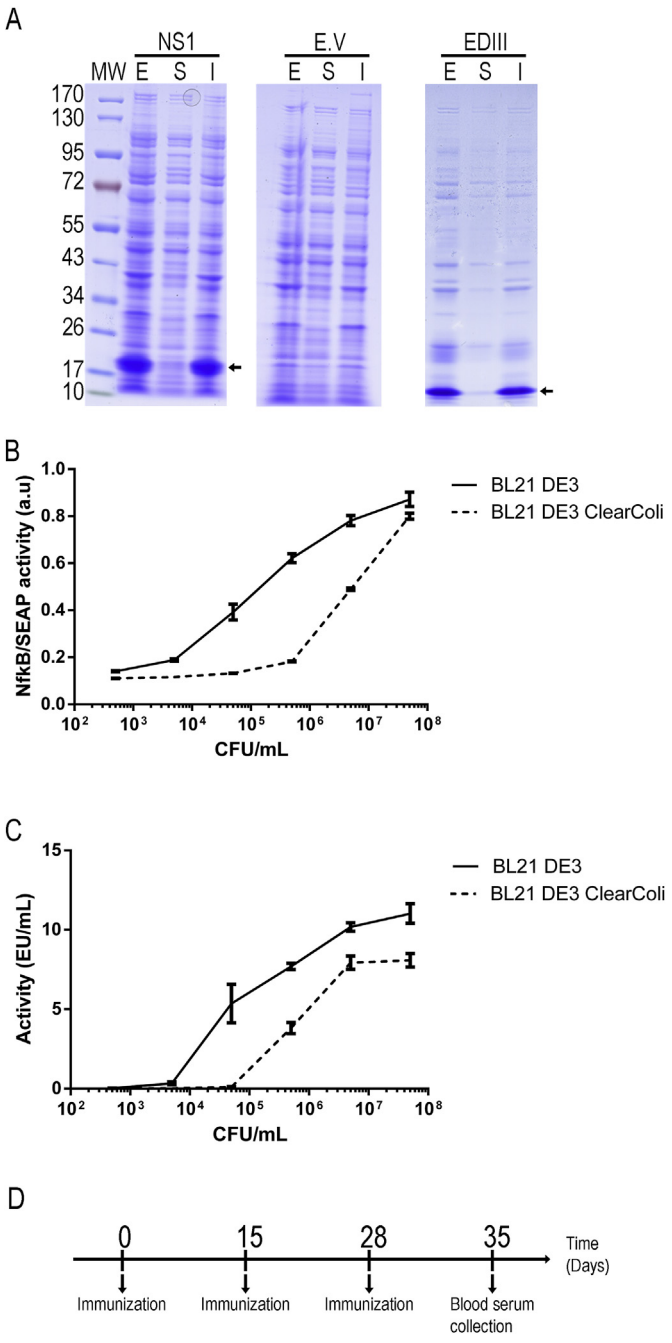

Fig. 1. Expression of recombinant ZIKV antigens in *E. coli* and immunization protocol in rat. **A**. ClearColi BL21(DE3) cells were transformed with pET28/ZIKV.rEDIII (EDIII) or pT28/ZIKV.$rNS1^{1-151}$ (NS1) or empty vector (E.V.). Proteins in crude cell lysates (E) were separated by SDS-PAGE followed by a Coomassie blue staining. The soluble (S) and insoluble (I) fractions of cell lysates were stained. The arrow heads indicate the position of $rNS1^{1-151}$ and rEDIII proteins. **B**. RAW-Blue cells were incubated with increasing doses of sonicated crude cell extracts from ClearColi BL21(DE3) or BL21(DE3). NF-κB activity was measured using QUANTI-blue assay. **C**. Endotoxic activity of sonicated BL21 Clear Coli or BL21DE3 was determined by LAL assay. **D**. Protocol of rat immunization. Crude extract of ClearColi BL21(DE3) cells containing ZIKV antigens were subcutaneously inoculated in adult female Wistar rats. The animals received two booster doses at days 15 and 28 after the first inoculation. Immune sera were collected one week after the last boosting inoculation.