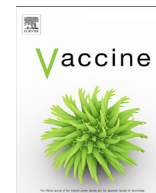
Vaccine xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

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



Short communication

Optimization of human papillomavirus (HPV) type 16 E7-expressing lactobacillus-based vaccine for induction of mucosal E7-specific IFNγ-producing cells

Atsushi Komatsu [a,b], Shizunobu Igimi [c,*], Kei Kawana [a,b,*]

[a] Department of Obstetrics and Gynecology, Nihon University School of Medicine, 30-1 Oyaguchi-kamimachi, Itabashi-ku, Tokyo 173-8610, Japan
[b] Department of Obstetrics and Gynecology, Faculty of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan
[c] Department of Applied Biology and Chemistry, Tokyo University of Agriculture, 1-1-1 Sakuragaoka, Setagaya-ku, Tokyo 156-8502, Japan

ARTICLE INFO

Article history:
Received 1 March 2018
Received in revised form 14 April 2018
Accepted 1 May 2018
Available online xxxx

Keywords:
Human papillomavirus (HPV)
Therapeutic HPV vaccine
Mucosal immunity
Cervical intraepithelial neoplasia (CIN)
Cervical cancer
Lactobacillus-based vaccine

ABSTRACT

Therapeutic HPV vaccine is an agent to induce E7-specific Th1 immune responses to treat cervical neoplasia (CIN2-3). Our previous clinical trial has demonstrated that oral administration of HPV16 E7-expressing *Lactobacillus casei* (*L. casei*), GLBL101c, resulted in the regression of HPV16-related CIN3. Here we examined optimization of the E7-expressing *L. casei* for induction of the mucosal immune responses to E7. Various doses of HPV16 E7 molecule were displayed on the *L. casei*. Immunization with E7-bound *L. casei* showed the induction of E7-specific mucosal IFNγ-producing cells was dependent on displayed E7-doses but saturated beyond 0.3 μg/10^8 cells. A new agent, *L. casei* with endogenous expression of E7 (IGMKK16E7), showed the optimal amount of displayed-E7. Immunization with IGMKK16E7 demonstrated 4-fold higher induction of E7-specific mucosal IFNγ-producing cells when compared with the former one. Our new system provided the optimal E7-expressing *L. casei* for displayed-E7 amount and induction of mucosal Th1 immune response.

© 2018 Published by Elsevier Ltd.

1. Introduction

HPV E7 is expressed strongly in the High-grade cervical intraepithelial neoplasia (CIN2-3) and cervical cancer, well-known to be immunogenic for human, and thereby may be a reliable target antigen for treatment of CIN2-3 and cervical cancer [1–3]. Indeed, many clinical trials have used HPV16 E7 as a vaccine antigen for therapeutic HPV vaccine for CIN2-3 [4–11].

Mucosal immune system in reproductive tract mucosa associates immunologically with gut-associated lymphoid tissue (GALT) [12,13]. This means that GALT can be the inductive site for mucosal immune system of uterine cervical mucosa.

We have generated a therapeutic HPV vaccine "GLBL101c", an attenuated HPV16 E7-expressing *Lactobacillus casei* (*L. casei*) [14–16]. The GLBL101c was administered orally to mice and elicited E7-specific IFNγ-producing cells into intraepithelial lymphocyte (IEL) derived from gut mucosa in immunized mice [16]. Our clinical data have revealed that 70% of CIN3 patients administered with GLBL101c had regression to CIN1-2, suggesting mucosal T cells recognizing E7 home to the cervical epithelium and exhibit Th1 immune responses against CIN lesion.

Although GLBL101c is generated by an expression system for *L. casei* strain, amount of E7 displayed on the cell surface has not been optimized so far. Here we addressed the correlation between amounts of E7 on the cell surface and the efficacy of induction of immune responses to E7. It led to the development of an optimized E7-expressing *L. casei*.

2. Materials and methods

2.1. Generation of the E7-bound and E7-expressing (IGMKK16E7) *L. casei*

To examine the optimal ratio for amount of E7 and lactobacilli, we established *L. casei* bound to various amounts of E7 on the cell surface. HPV16-derived E7 gene was modified by insertion of point mutations into the Rb-binding site [16], named mutated E7Rb. We prepared purified HPV16-derived mutated E7Rb proteins fused with an anchor protein, cA, derived from *Lactococcus lactis*. Various

* Corresponding authors at: Department of Obstetrics and Gynecology, Nihon University School of Medicine, 30-1 Oyaguchi-kamimachi, Itabashi-ku, Tokyo 173-8610, Japan (K. Kawana).

E-mail addresses: s3igimi@nodai.ac.jp (S. Igimi), kkawana-tky@umin.org (K. Kawana).

https://doi.org/10.1016/j.vaccine.2018.05.009
0264-410X/© 2018 Published by Elsevier Ltd.

Please cite this article in press as: Komatsu A et al. Optimization of human papillomavirus (HPV) type 16 E7-expressing lactobacillus-based vaccine for induction of mucosal E7-specific IFNγ-producing cells. Vaccine (2018), https://doi.org/10.1016/j.vaccine.2018.05.009

amounts of cA-E7 purified protein were conjugated with our *L. casei* strain, IGM393.

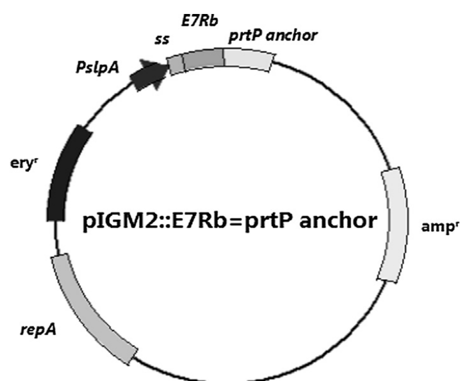
We generated a new-generation recombinant E7-expressing *L. casei*, named IGMKK16E7, as following. Open reading frame of HPV16-derived mutated E7Rb gene was fused with an anchor gene, a proteinase sequence of *L. casei*: prtP anchor, at the C-terminus of mutated E7Rb (Fig. 1). The plasmid construction of expression vector was transduced into our *L. casei* strain, IGM394. The E7-expressing *L. casei* was attenuated by heating.

2.2. Immunization protocols

GLBL101c, a prior E7-expressing *L. casei*, was kindly gifted from BioLeaders Corp. (Korea) and AnGes MG, Inc. (Japan). GLBL101c is a *L. casei* 525 strain which expresses mutated E7Rb by an expression plasmid, pKV-Pald::pgsA = E7Rb [15]. E7-bound and E7-expressing *L. casei* were attenuated. 1.0 mg of powder weight (=1 × 10^5 cells) / head of GLBL101c, E7-bound or E7-expressing *L. casei* were administered with C57BL/6 mice four times at weeks 1, 2, 4, and 6 as previously reported [17]. All inoculums were administered once a day for five days each week via intra-gastric tube after 3 h of fasting. Intestinal intraepithelial lymphocytes (IEL) were collected from immunized mice. Approximately 5–10 × 10^6 intestinal mucosal lymphocytes were obtained from an individual mouse.

2.3. Enzyme linked immunosorbent assay (ELISA) and flow cytometry analysis

The contents of mutated E7Rb at protein level in E7-bound and E7-expressing *L. casei* and GLBL101c were measured using ELISA. The monoclonal mouse anti-HPV16 E7 antibodies were used for the capture and detection antibodies (Abcam plc, UK). These *L. casei* were incubated with an anti-HPV16 E7 antibody (Santa Cruz Biotechnology, USA) using a FACS.



PslpA	promoter sequence of *Lactobacillus brevis* ATCC1559 S-layer protein
ss	secretion signal of *L. brevis* ATCC1559 *prtP gene*
E7Rb	Human papillomavirus E7 gene
anchor	proteinase sequence of *Lactobacillus casei*
amp^r	ampicillin resistant gene of plasmid pGEM-3
ery^r	erythromycin resistant gene of *Staphylococcus aureus* plasmid pE194

Fig. 1. Expression vector construct of IGMKK16E7, a new generation of E7-expressing *L. casei*. E7Rb means full open reading frame of HPV16 E7 gene with three point mutations at the Rb-binding site as previously reported [20]. The plasmid DNA was transduced into the L. casei strain, IGM394, to generate IGMKK16E7.

2.4. ELISPOT assay

ELISPOT assay was performed according to modified protocol as reported previously [16]. In brief, 50 μL of intestinal IEL (1 × 10^5 cells) were incubated for 48 h at 37 °C with antigen-presenting cells in 96-well ELIIP plate (Millipore USA). 10 μL of a synthesized peptide corresponding to a reported CTL epitope for C57BL/6 (H-2b) mice were added to wells coated to anti-mouse IFNγ monoclonal antibodies.

3. Results

3.1. E7-specific mucosal immune responses by E7-bound L. casei

To decide the optimized dose of E7 displayed on the *L. casei* for induction of mucosal immunity, we generated *L. casei* displaying various amounts of E7 molecule. HPV16 E7 whole protein fused with a *Lactococcus lacti*-derived anchor protein (cA = E7) was immobilized on the cell surface by anchoring. 0.03, 0.1, 0.3, 1.0 and 3.0 μg/10^8 cells of cA = E7 purified protein were conjugated with *L. casei*. The amounts of E7 displayed on the cells was analyzed quantitatively by flow cytometry using anti-E7 antibody (Fig. 2A). The amount of displayed-E7 was saturated at concentration of 1.0 μg/10^8 cells since the histogram of 1.0 and 3.0 μg/10^8 cells were completely overlapped. Therefore, 1.0 μg of E7 appeared to be maximum amount of E7 that can be displayed on 10^8 cells of *L. casei* by our system.

E7-bound *L. casei* (E7: 0.03, 0.1, 0.3, 1.0, and 3.0 μg/10^8 cells) were administered orally with mice. The intestinal IELs were used for ELISPOT assay to detect E7-specific mucosal IFNγ-producing cells elicited by oral immunization with E7-bound *L. casei* (Fig. 2B). Numbers of E7-specific IFNγ-producing cells in the intestinal IELs were increased in a manner dependent on amounts of displayed-E7 up to 0.3 μg/10^8 cells ($p = 0.0195$) while saturated beyond 0.3 μg/10^8 cells ($p = 0.65$). The mucosal E7-specific cellular immune responses was clearly associated with the amounts of displayed-E7 of vaccine antigen.

3.1.1. Generation and characterization of E7-expressing L. casei

We developed a novel expression and display system and generated an *L. casei* (IGMKK16E7) for optimization of amounts of displayed-E7 (Fig. 1), which are completely different from those of GLBL101c. ELISA for E7 protein levels demonstrated that E7 content of IGMKK16E7 was almost equal to that of 0.3 μg/10^8 cells of E7-bound *L. casei* and barely greater than that of GLBL101c (data not shown). On the other hand, the amount of displayed-E7 was evaluated by flow cytometry using anti-E7 antibody. The histograms of mock, GLBL101c, E7-bound *L. casei* (0.1 μg/10^8 cells), and IGMKK16E7 were indicated by No. 1, 2, 3, and 4 lines, respectively (Fig. 3A). Mean fluorescence intensity (MFI) of E7 positivity of IGMKK16E7 was clearly higher than those of GLBL101c and E7-bound *L. casei* (0.1 μg/10^8 cells), indicating indicated the amount of displayed E7 molecules on IGMKK16E7 was much higher than that on GLBL101c. 0.3 μg/10^8 cells of E7-bound *L. casei* was regenerated by endogenous E7 gene expression as an E7-expressing *L. casei*, IGMKK16E7.

3.1.2. Comparison in E7-specific mucosal immune responses between IGMKK16E7 and GLBL101c

E7-speific mucosal immune responses by oral immunization were compared between IGMKK16E7 and GLBL101c using mice experiment. To examine the dose-dependency of IGMKK16E7 for induction of E7-specific mucosal immune responses, we also prepared various doses of IGMKK16E7 (0.1 mg, 0.3 mg, 1.0 mg and

Download English Version:

https://daneshyari.com/en/article/8485616

Download Persian Version:

https://daneshyari.com/article/8485616

Daneshyari.com