Enhanced humoral and cellular immunity using HPV quadrivalent recombinant vaccine formulated with the modified lipopolysaccharide/ bacterial DNA fragments/aluminium salt combination compared to aluminium salt

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Directed by Professor Dong Jae Cho

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Ga Hyun Son

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<ABSTRACT>

Enhanced humoral and cellular immunity using HPV quadrivalent recombinant vaccine formulated with the modified lipopolysaccharide/bacterial DNA fragments/ aluminium salt combination compared to aluminium salt

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Developing efficient and safe adjuvants for use in human vaccines remains both a challenge and a necessity. CIA07 is a immunostimulatory agent composed of *E.coli* DNA fragments and modified lipopolysaccharide (LPS) lacking the lipid A moiety. In this study, we investigated adjuvant activity of CIA07 using HPV L1 VLPs as the immunogen. Mice were immunized intramuscularly two times at 3-week intervals with HPV 6/11/16/18 L1 VLP vaccine alone or in combination with bacterial DNA fragments, modified LPS or CIA07, and immune responses were assessed. Modified LPS and CIA07 adjuvanted formulation showed significantly higher titers of HPV 16/18 L1 VLPs specific antibodies than aluminium salt. HPV 16/18 L1 VLPs specific IgG isotype titers were measured. Modified LPS adjuvanted formulation stimulated the IgG1 antibody response effectively, but did not induce a significant increase in the IgG2a antibody response. On the other hand, CIA07 adjuvanted formulation induced significantly higher titers of HPV 16/18 L1 VLPs specific IgG1 as well as IgG2a antibodies at 28-day

post-immunization II. Furthermore, the ratio of IgG2a to IgG1 antibody titers in mice administered with CIA07 adjuvanted formulation was higher compared to aluminium salt at 28-day post-immunization II, indicating that CIA07 could stimulate higher Th1-type immune responses to HPV 16/18 L1 VLPs.

These data demonstrated that CIA07 adjuvanted formulation has the ability to induce higher humoral and cellular immune responses in mice when compared with the aluminium salt, and support the role of the CIA07 as an effective adjuvant to the HPV quadrivalent recombinant vaccine.

Key words : adjuvant, HPV L1 VLPs, antibody response, cellular immune response

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I. INTRODUCTION

Cervical cancer is one of the most common malignancies in women world-wide and virtually all of cervical cancers are associated with human papillomavirus (HPV) infection.[1,2] Approximately 40 different types of HPVs that infect the mucosal epithelium of the genital tract have been identified, of which 15 HPV types have been classified as 'high risk' or 'oncogenic' as they are associated with the development of cervical cancer.[3-7]

There are two prophylactic HPV vaccines being commercially available. Gardasil® is the only quadrivalent (HPV types 6, 11, 16, and 18) vaccine, and is the first vaccine to be approved for use in the prevention of anogenital cancers, precancers or warts related to HPV infection. The quadrivalent HPV vaccine consists of a mixture of four types of viral DNA-free virus-like particles (VLPs) derived from the L1 capsid proteins of HPV types 6, 11, 16, and 18, and uses aluminium salts as an adjuvant.[8] Adjuvants are required to assist vaccines to induce potent and persistent immune responses, with the additional benefits that less antigen and fewer injections are needed.

Aluminium salts, the most commonly used adjuvant in vaccines licensed for

human use, predominantly induce antibody responses, but is poorly effective in inducing cell mediated responses essential for the treatment of viral infections.[9,10] Therefore, the past 10 years new adjuvants systems have been designed to potentiate both specific antibody and cellular immune responses to vaccination.

Bacterial DNA is recognized by cells in the innate immune system through the Toll-like receptor (TLR) 9, which potently stimulates both innate and adaptive immune responses.[11-13] CpG motifs in bacterial DNA are responsible for this immunostimulatory activity.[14] Recent report show that *Escherichia coli* DNA activates macrophages more potently than CpG-containing oligonucleotides. This enhanced immune stimulatory activity is attributed to the length of DNA.[15] Lipopolysaccharide (LPS), a component of the outer membrane of Gram-negative bacteria, is a potent activator of various immune cells including neutrophils, macrophages, and CD4 and CD8 T cells that infiltrate tumors, and endothelial cells through interactions with one or more Toll-like receptors.[16-19] LPS stimulates the secretion of cytokines such as tumor necrosis factor-α (TNF-α), inferferon-γ (IFN- γ), interleukin (IL)-1β, and IL-6. Because of its strong immunostimulating activity, LPS has been extensively investigated as an antitumor therapy, but its clinical application in humans has been limited by its high toxicity.[20-25]

The immunostimulatory activities of both bacterial DNA fragments and modified lipopolysaccharide analyzed previously.[26] *Escherichia coli* genomic DNA fragments between 0.5 and 2.0 kb in size displayed the highest adjuvant activity in mice. DNA fragments were combined with lipopolysaccharide that was alkaline-hydrolyzed to remove lipid A. The resulting mixture, designated CIA07, exhibited greater immunostimulatory activity in vitro in a mouse bladder cancer model than the current immunotherapeutic agent, Bacillus Calmette-Guerin (BCG). Moreover, CIA07 did not cause any apparent adverse reactions in animals, which has led to limitations in the use of

lipopolysaccharide as a vaccine adjuvant. These findings collectively support the potential of CIA07 as a novel immunomodulatory agent.[26]

The objective of the present investigation was to evaluate the immune profile induced by quadrivalent recombinant vaccine formulated with the modified lipopolysaccharide/bacterial DNA fragments/aluminium salt combination compared to aluminium salt in mice.

II. MATERIALS AND METHODS

1. Reagents

Gardasil® obtained from Merck & Co. was used for immunization of mice. HPV16 L1 and HPV18 L1 VLPs used as ELISA antigen were expressed in yeast. bacterial DNA fragments, modified LPS and CIA07 composed of bacterial DNA fragments and modified LPS were prepared as described by Cho *et al.* [26]

2. Preparation of adjuvant CIA07

CIA 07, composed of bacterial DNA fragments and modified lipopolysaccharide, was prepared as described by Cho et al., 2006.[26] Briefly, lipopolysaccharide containing a short carbohydrate chain was isolated from *E.coli*, and alkaline-hydrolyzed for the removal of lipid A moieties, followed by ethanol precipitation. The resulting modified lipopolysaccharide was quantitated using the 2-keto-3-deoxyoctonate assay.[27] The reduced size of modified lipopolysaccharide was confirmed on a silver-stained sodium dodecyl sulphate (SDS)-polyacrylamide gel.

Bacterial chromosomal DNA was extracted from E.coli using phenol/chloroform/isoamyl alcohol (25: 24: 1) and precipitated with ethanol. The DNA pellet was dissolved in water, fragmented by sonication and treated with Triton X-114 to remove contaminating endotoxins,[26] followed by organic extractions and ethanol precipitation. The endotoxin content of DNA was determined using the LAL assay kit and confirmed to be <0.1 ngmg-1 bacterial DNA. DNA fragment sizes were analyzed on an agarose gel stained with ethidium bromide, and confirmed using a Bioanalyzer 2100 with the DNA 7500 LabChip (Agilent Technologies, Palo Alto, CA). Finally, CIA07 was prepared

by mixing bacterial DNA fragments and modified lipopolysaccharide at a ratio of 100:1.

‘

3. Immunization of mice with HPV 6/11/16/18 L1 VLP vaccine

Six-week-old male Balb/c mice were purchased from Japan SLC (Hamamatsu, Japan). Animals were randomly assigned into groups of five. Groups of five male Balb/c mice were immunized intramuscularly twice at a 3-week interval with vaccine antigen in the presence of adjuvants. The amounts of the antigen and adjuvants used for immunization were as follows: 1/20 human dose (25 ul) of HPV 6/11/16/18 L1 VLP vaccine alone or combined with 50 μg bacterial DNA fragments, 0.5 μg modified LPS or 50 μg CIA07. Control mice were given saline.

4. Determination of serum antibody titers specific for HPV 16/18 L1 VLPs

Blood was collected from the animals at 7- and 28-day post-immunization II by a heart puncture, allowed to clot at 4℃ overnight and centrifuged. Sera were divided into aliquots, and maintained at – 70℃ until use.

HPV L1-specific antibody titer in immunized sera was determined by end-point dilution ELISA. Ninety six-well microtiter plates were coated overnight with 100 μℓ of 1 μg/ml HPV L1 in 50mM carbonate buffer, pH 9.6 at 4℃. The plates were blocked with 300 μℓ of 1% BSA in PBS at 37℃ for 1 hr. Following washes with PBS-0.05% tween 20, serial 2-fold dilutions of sera in PBS containing 1% BSA were made across the plate and plates were incubated at 37℃ for 2 hr. The plates were then washed with PBST and incubated with HRP-conjugated goat anti-mouse IgG, IgG1 or IgG2a antibodies followed by

colorimetric detection using TMB as the color substrate. A volume of 1 N H_2SO4 (100 μl) was added to terminate the reaction, and the absorbance at 450 nm was measured using an microplate reader. To exclude assay variations, anti-VLP L1 hyperimmune serum was included in each ELISA plate. End-point titers were defined as the highest serum dilutions that resulted in an absorbance value twice as high as that of nonimmune serum with a cutoff value of 0.1, and were expressed as the group geometric means ± SD of five mice.

5. Statistical analysis

Statistical analyses of samples were performed using SPSS version 12.0 (SPSS Inc., Chicago IL, USA) Geometric mean antibody titers (GMT) with 95% CI were calculated using log10-transformed individual titers and expressed as the anti-log of the mean. The statistical significance of differences between groups was determined with Student's t-test. $P<0.05$ was considered to be significant.

III. RESULTS

1. Humoral immune responses induced by adjuvants.

Balb/c mice were intramuscularly immunized two times at 3 week intervals with 25 ul of HPV 6/11/16/18 L1 VLP vaccine alone or combined with bacterial DNA fragments, modified LPS or CIA07. Sera were obtained at 7- and 28-day post-immunization II, and HPV16/18 L1 VLPs-specific total IgG antibody titers were determined by end point dilution ELISA, and the geometric mean antibody titers were calculated.

After two intramuscular vaccinations, the modified LPS and CIA07 adjuvanted formulation induced significantly higher titers of HPV 16 (6.9-fold and 6.9-fold, $p<0.001$ and $p<0.001$, respectively) and HPV 18 (2.3-fold and 4.0-fold, $p<0.05$ and $p<0.05$, respectively) L1 VLPs specific antibodies than aluminium salt only at 7-day post-immunization II. This difference persisted up to 28-day post-immunization II. The groups vaccinated with the formulation containing modified LPS and CIA07 demonstrated significantly higher anti-HPV 16 and HPV 18 L1 VLPs antibody responses (Modified LPS : 9.2-5.3-fold, $p<0.01$, CIA07 : 6.9-8.0-fold, $p<0.01$, respectively) than aluminium salt only at 28-day post-immunization II (Fig. 1,2).

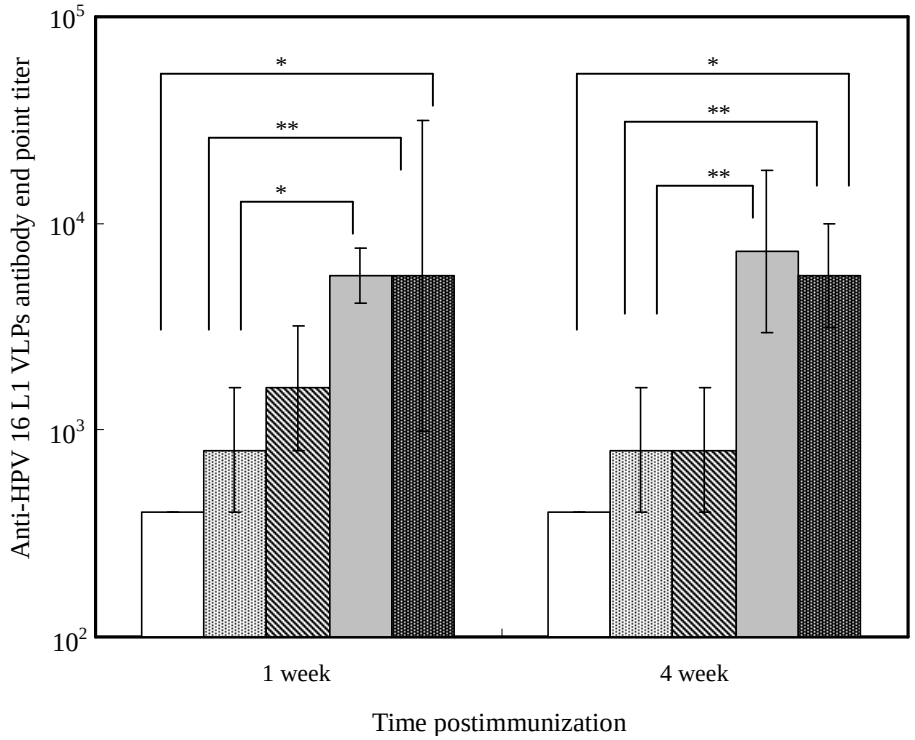

Figure 1. Total Serum IgG antibody titers specific for HPV16 L1 VLPs

Balb/c mice were immunized intramusculary twice at a 3-week interval with 25 ul of HPV 6/11/16/18 L1 VLP vaccine alone or combined with bacterial DNA fragments, modified LPS or CIA07. Sera were obtained at 7- and 28-day post-immunization II, and HPV 16 L1 VLPs specific serum total IgG antibody titers were measured by end-point dilution ELISA. Data are presented as geometric means± SD of ELISA titers obtained from five mice in each group.

Symbols: □, control; ▩, HPV 6/11/16/18 L1 VLP vaccine alone; ▨, adjuvant with bacterial DNA fragments; ▤, adjuvant with modified LPS; ■, adjuvant with CIA07. *$p < 0.001$; **$p < 0.01$

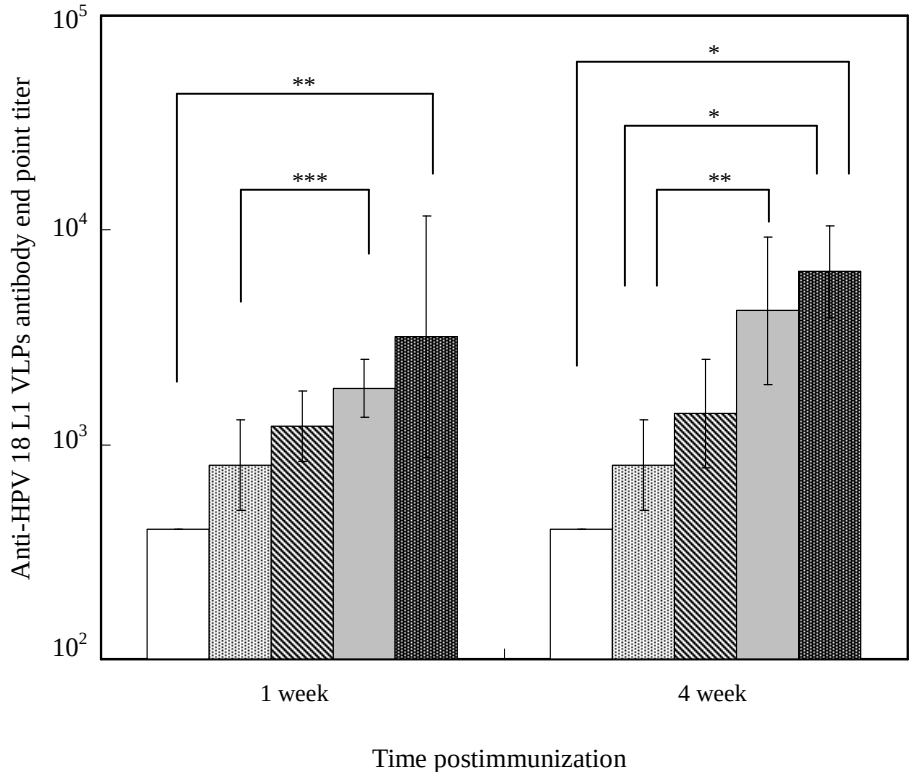

Figure 2. Total Serum IgG antibody titers specific for HPV18 L1 VLPs

Balb/c mice were immunized intramusculary twice at a 3-week interval with 25 ul of HPV 6/11/16/18 L1 VLP vaccine alone or combined with bacterial DNA fragments, modified LPS or CIA07. Sera were obtained at 7- and 28-day post-immunization II, and HPV 18 L1 VLPs specific serum total IgG antibody titers were measured by end-point dilution ELISA. Data are presented as geometric means± SD of ELISA titers obtained from five mice in each group.

Symbols: □, control; ▩, HPV 6/11/16/18 L1 VLP vaccine alone; ▨, adjuvant with bacterial DNA fragments; ▩, adjuvant with modified LPS; ■, adjuvant with CIA07. *$p < 0.001$; **$p < 0.01$; ***$p < 0.05$

No apparent systemic adverse reactions (i.e. change in body weight and mobility or ruffling of fur) were observed in either strain administered CIA07, and no swelling or ulceration was evident at the injection site.

2. Th type immune responses induced by adjuvants

In mice, IgG isotype distribution is often used as an indicator for evaluating the Th bias of immune responses. High IgG2a/IgG1 ratios signify a Th1-biased immune response. HPV16 and HPV18 L1 VLPs specific IgG isotype titers were measured in mice to determine the Th type of immune responses induced by adjuvants. Groups of five Balb/c mice were intramuscularly immunized two times at 3-week intervals with 25 ul of HPV 6/11/16/18 L1 VLP vaccine alone or combined with bacterial DNA fragments, modified LPS or CIA07, and serum IgG isotype titers were assessed by ELISA (Fig. 3,4). The ratio of IgG2a to IgG1 antibody levels in mice immunized with HPV 6/11/16/18 L1 VLP vaccine alone and combined with adjuvants was calculated for each group (Table 1).

Modified LPS stimulated the IgG1 antibody response effectively. Modified LPS adjuvanted formulation induced significantly higher titer of HPV 16 and HPV 18 L1 VLPs specific IgG1 antibodies (5.3-fold, $p<0.01$ and 4.6-fold, $p<0.05$, respectively) as compared to aluminium salt only at 7-day post-immunization II. A similar observation was made at 28-day post-immunization II (3.5-fold, $p<0.05$ and 6.1-fold, $p<0.05$, respectively). At 1 week after the two injections, the group vaccinated with the CIA07 adjuvanted formulation did not display significantly higher anti HPV 16 and HPV 18 L1 VLPs specific IgG1 antibody responses (3.0-fold, $p=0.27$ and 3.5-fold, $p=0.17$, respectively). However, CIA07 adjuvanted formulation induced significantly higher titers of HPV 16 and HPV 18 L1 VLPs specific IgG1 antibodies at 28-day post-immunization II (3.0-fold, $p<0.05$ and 3.5-fold, $p<0.05$, respectively) (Fig. 3).

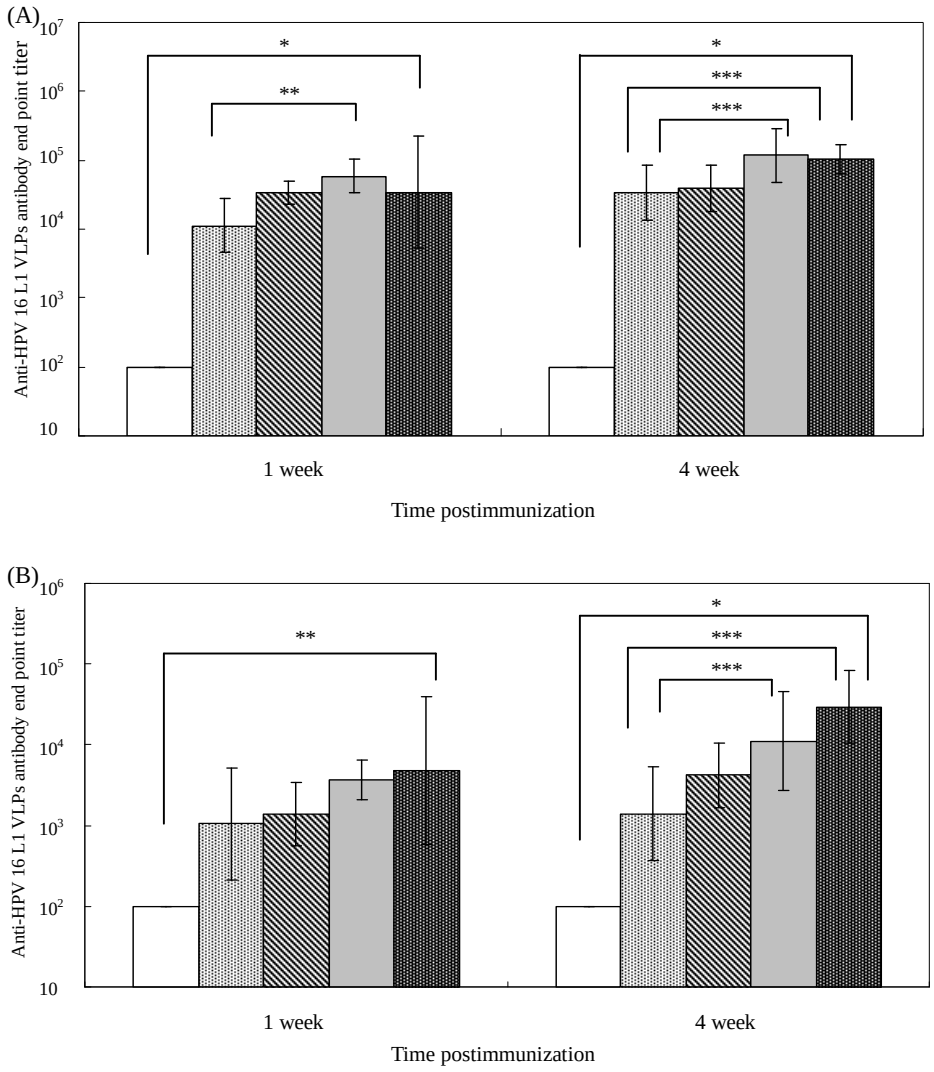

Figure 3. Serum IgG isotype antibody titers specific for HPV 16 L1 VLPs. (A) IgG1 antibody titer (B) IgG2a antibody titer. Data are presented as geometric means± SD of ELISA titers obtained from five mice in each group. Symbols: □, control; ▩, HPV 6/11/16/18 L1 VLP vaccine alone; ▨, adjuvant with bacterial DNA fragments; ▢, adjuvant with modified LPS; ■, adjuvant with CIA07. *$p < 0.001$; **$p < 0.01$; ***$p < 0.05$

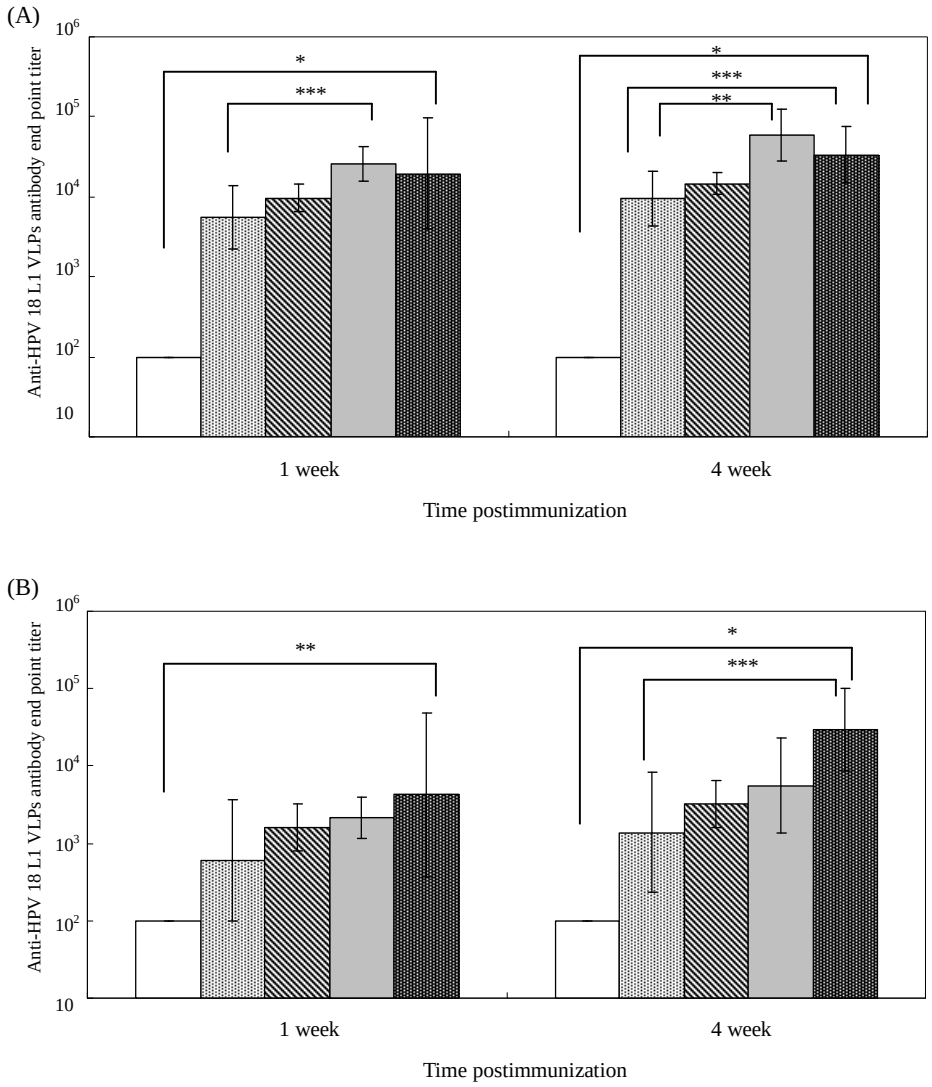

Figure 4. Serum IgG isotype antibody titers specific for HPV18 L1 VLPs. (A) IgG1 antibody titer (B) IgG2a antibody titer. Data are presented as geometric means± SD of ELISA titers obtained from five mice in each group. Symbols: □, control; ▩, HPV 6/11/16/18 L1 VLP vaccine alone; ▨, adjuvant with bacterial DNA fragments; ▢, adjuvant with modified LPS; ■, adjuvant with CIA07. *$p < 0.001$; **$p < 0.01$; ***$p < 0.05$

The group vaccinated with modified LPS did not display an effective increase of the IgG2a antibody response as that of the IgG1 antibody. Modified LPS adjuvanted formulation stimulated significantly higher titer of HPV 16 L1 VLPs specific IgG2a at 28-day post-immunization II, and did not induce significantly higher titers of HPV 18 L1 VLPs specific IgG2a antibodies at all time points compared with aluminium salt. On the other hand, The CIA07 adjuvanted vaccine formulation induced significantly higher titers of HPV 16 and HPV 18 L1 VLPs specific IgG2a antibodies at 28-day post-immunization II.

The ratio of IgG2a to IgG1 antibody levels in mice immunized with HPV 6/11/16/18 L1 VLPs vaccine alone and combined with adjuvants was showed in table 1. The ratios of HPV 16 L1 VLPs specific IgG2a to IgG1 in mice administered with aluminium salt were 0.32 ± 0.43 at 7-day and 0.05 ± 0.04 at 28-day post-immunization II, indicating predominant activation of Th2-type immune response. The group injected with CIA07 adjuvanted formulation displayed high IgG isotype titers, and the ratios were 0.20 ± 0.18 at 7-day and 0.41 ± 0.36 at 28-day post-immunization II. The ratios of HPV 18 L1 VLPs specific IgG2a to IgG1 in mice administered with aluminium salt were 0.42 ± 0.52 at 7-day and 0.47 ± 0.86 at 28-day post-immunization II, suggesting of Th2-biased immune response. The group injected with CIA07 adjuvanted formulation displayed higher IgG isotype titers, and the ratios of HPV 18 L1 VLPs specific IgG2a to IgG1 were 0.45 ± 0.50 at 7-day and 1.30 ± 1.52 at 28-day post-immunization II.

Our data indicated that CIA07 formulation stimulated higher Th1-type immune responses to HPV 16/18 L1 VLPs compared to aluminium salt at 28-day post-immunization II.

Table 1. Ratios of IgG2a/IgG1 antibody titers in mice immunized with HPV 6/11/16/18 L1 VLP vaccine in combination with adjuvants.

	IgG2a/IgG1 ratio			
	HPV16 L1 VLPs (n=5)		HPV18 L1 VLPs (n=5)	
Adjuvant	1 week	4 week	1 week	4 week
HPV 6/11/16/18 L1 VLP vaccine	0.32 ± 0.43	0.05 ± 0.04	0.42 ± 0.52	0.47 ± 0.86
HPV 6/11/16/18 L1 VLP vaccine + bacterial DNA	0.05 ± 0.04	0.17 ± 0.19	0.19 ± 0.08	0.28 ± 0.20
HPV 6/11/16/18 L1 VLP vaccine + modified LPS	0.08 ± 0.05	0.28 ± 0.41	0.10 ± 0.05	0.28 ± 0.41
HPV 6/11/16/18 L1 VLP vaccine + CIA07	0.20 ± 0.18	0.41 ± 0.36	0.45 ±0.50	1.30 ± 1.52

Data : mean ± SD

IV. DISCUSSION

In this study, the immunostimulatory activity was investigated of bacterial DNA fragments, modified LPS and CIA07 as adjuvant against HPV 6/11/16/18 L1 VLP vaccine. The results demonstrated that CIA07 adjuvanted formulation is capable of inducing higher humoral and cellular immune responses when compared with the aluminium salt only formulation.

The adjuvant activities were compared. Aluminium salt and bacterial DNA adjuvanted formulation displayed similar adjuvant activities. In contrast, modified LPS and CIA07 adjuvanted formulations exerted significantly enhanced serum antibody responses to HPV 6/11/16/18 L1 VLP vaccine in Balb/c mice.

Toll-like receptors (TLR) are among the pattern-recognition receptors, and have been shown to have a crucial role in the early steps of the immune response to infection. TLR sensing of their natural or synthetic agonists can orientate the immune response towards a Th1- or Th2- cell response, and promote regulatory T cell activity.[28-32] In particular, LPS agonists and unmethylated CpG oligonucleotides induce Th1 responses after sensing by TLR4 and TLR9, respectively.[33] Different TLR agonists can synergize and/or balance each other's immunomodulatory activity. Synergy was observed with combinations such as TLR2-TLR4, TLR3-TLR4-TLR7/8, TLR4-TLR9, and so on.[34-36]

Recent vaccines concentrate on introducing a cellular Th1 response in addition to an antibody response to increase vaccine efficacy against targets such as certain viruses, for instance, through TLR4 or TLR9 agonists. Previous study demonstrated that TLRs control Th-cell orientation and the antibody isotypes that are produced, as seen by an overall bias towards a Th2 response in the TLR-signalling deficient mice.[37] This TLR-signalling deficient mice showed a marked decrease in Th1-cell-linked IgG3 levels and less pronounced decrease in

IgG2a and IgG2b. Therefore, associating TLR-dependent and TLR-independent adjuvants could be beneficial, by triggering different and potentially synergistic pathways: TLR-independent 'classical' adjuvants such as alumimium salt would increase the global level of the immune response, and TLR agonists would modulate its quality (Th1/Th2-cell bias). The combination of CIA07/aluminium salt used in present study could be beneficial in that it is the combination of TLR-independent and TLR-dependent adjuvants, where TLR-dependent adjuvants recognize both TLR4 and TLR9 by containing bacterial DNA and modified LPS.

There were previous studies of adjuvant effect of CIA07 on the immune response to hepatitis B virus surface antigen and influenza subunit vaccine.[38,39] Bacterial DNA fragment in previous studies acts synergistically with modified LPS to stimulate antibody responses. Thus, CIA07, a combination of those two components, induced most effective stimulation of humoral immune response. Furthermore, those studies demonstrated that CIA07 exhibited high IgG1 as well as IgG2a antibody titers to HBsAg and influenza subunit vaccine (IgG2a/IgG1>1.3 and 0.66-1.0, respectively), suggesting that CIA07 is able to effectively induce both cellular and humoral immnune responses.

On the other hand, the groups vaccinated with CIA07 adjuvanted formulation in this study displayed significantly higher humoral immune responses, but synergy was not observed between bacterial DNA and modified LPS.

The present study also showed that the ratio of IgG2a to IgG1 antibody titers in mice administered with CIA07 adjuvanted formulation was higher at 28-day post-immunization II when compared with aluminium salt which is known to predominantly induce antibody responses. However, the IgG2a/IgG1 ratio was relatively low in comparison with that from the previous studies. Several explanations were possible regarding these findings that were different from the previous reports.

Both antigen and immunostimulant should retain their native conformation

ability to interact with their ligands.[40,41] In this respect, the antigen-adjuvant couple can be crucial, as the antigen itself might increase or counteract adjuvant efficacy or affect that induced Th1-cell bias. Therefore, the efficacy of the CIA07 and quality of immune responses could be affected by HPV L1 VLPs antigen unlike the results from previous studies.[38,39]

Further studies such as the assessment of interferon-γ production in spleens may be required to confirm the induction of Th1-type cellular response by adjuvants to HPV 6/11/16/18 L1 VLP vaccine.

V. CONCLUSION

The results of the present study showed that Modified LPS and CIA07 adjuvanted formulation induced significantly higher titers of HPV 16/18 L1 VLPs specific antibodies than aluminium salt. CIA07 adjuvanted formulation also induced significantly higher titers of HPV 16/18 L1 VLPs specific IgG2a and IgG1 antibodies at 28-day post-immunization II. The ratio of IgG2a to IgG1 antibody titers in mice administered with CIA07 adjuvanted formulation was higher when compared with aluminium salt at 28-day post-immunization II, indicating that CIA07 could stimulate Th1-type as well as Th2-type immune responses to HPV 16/18 L1 VLPs.

These data demonstrated that CIA07 adjuvanted formulation has the ability to induce higher humoral and cellular immune responses to the HPV quadrivalent recombinant vaccine compared to aluminium salt in mice, and support the role of the CIA07 as an effective adjuvant.

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< ABSTRACT(IN KOREAN)>

HPV 4가 백신에서 변형된 lipopolysaccharide/bacterial DNA fragments/aluminium salt 복합체가 보조제로서 면역 반응에 미치는 영향

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손가현

백신 보조제는 면역 반응을 향상시키고 백신에 사용되는 항원의 양을 줄이는데 도움이 되는 물질로 보다 효과적이고 안전한 보조제 (adjuvant) 를 개발하는 것은 백신 연구에서 중요하고 어려운 과제이다. *E.coli* DNA의 일부분과 변형된 lipopolysaccahride (LPS)의 복합체를 CIA07이라고 명명하였을 때 본 연구에서는 HPV 4가 백신에서 보조제로 aluminium salt만을 사용하였을 때에 비하여 CIA07을 첨가하였을 때의 효과를 알아보고자 한다.

쥐를 5마리씩 5군으로 하여 각각 다음과 같이 3주 간격으로 2회 근육주사 하였다. 1군은 대조군으로 생리식염수, 2군은 HPV 4가 백신, 3군은 HPV 4가 백신과 세균 DNA fragments, 4군은 HPV 4가 백신과 변형된 LPS, 5군은 HPV 4가 백신과 CIA07을 근육주사 하였고 두 번째 백신 주사 후 1주와 4주 후에 면역반응을 측정하였다.

보조제로 변형된 LPS와 CIA07을 첨가한 경우 aluminium salt만을 사용한 경우보다 HPV 16/18 L1 VLPs에 대한 항체(IgG)가 유의하게 증가하였다. 또한 HPV 16/18 L1 VLPs specific IgG isotype을 측정하였을 때 CIA07을 보조제로 첨가하였을 경우 aluminium salt만을 사용한 경우보다 두 번째 백신 주사 4주 후 측정한 HPV 16/18 L1 VLPs에 대한 IgG1 와 IgG2a 항체는 모두 증가하였다. 그러나 변형된 LPS를 보조제로 사용한 경우 IgG1은 효과적으로 증가시키는 반면 IgG2a는 증가시키지 못하였다. HPV 16/18 L1 VLPs specific IgG2a/IgG1 ratio을 계산한 결과 두 번째 백신 주사 4주 후에, CIA07을 보조제로 첨가한 경우 aluminium salt만을 사용하였을 경우보다 높은 수치를 보여 CIA07을 첨가한 보조제를 사용하였을 경우 Th1형 면역반응을 더 효과적으로 유도할 수 있다는 것을 시사하였다.

이번 연구 결과를 종합하여 볼 때 CIA07을 HPV 4가 백신의 보조제로 첨가할 경우 aluminium salt만을 사용하였을 경우보다 체액성 및 세포성 면역 반응을 보다 향상시킬 수 있어 효과적인 면역 보조제로서의 역할을 할 수 있으리라 기대된다.

핵심되는 말 : 보조제 (adjuvant), HPV L1 VLPs antigen, 항체 반응, 세포성 면역 반응