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The Production and Immunogenicity of Human Papillomavirus Type 58 Virus-like Particles Produced in *Saccharomyces cerevisiae*[§]

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Human papillomavirus (HPV) is the cause of most cases of cervical cancer. HPV type 58 (HPV58) is the second most frequent cause of cervical cancer and high-grade squamous intraepithelial lesions (HSIL) in Asia and South / Central America, respectively. However, there is no vaccine against HPV58, although there are commercially available vaccines against HPV16 and 18. In this study, we produced HPV58 L1 protein from *Saccharomyces cerevisiae*, and investigated its immunogenicity. We first determined the optimum period of culture for obtaining HPV58 L1. We found that a considerable portion of the HPV58 L1 resulting from 48 h culture cannot be recovered by purification, while the HPV58 L1 resulting from 144 h culture is recovered efficiently: the yield of HPV58 L1 finally recovered from 144 h culture was 2.3 times higher than that from 48 h culture, although the production level of L1 protein from 144 h culture was lower than that from 48 h culture. These results indicate that the proportion of functional L1 protein from 144 h-cultured cells is significantly higher than that of 48 h-cultured cells. The HPV58 L1 purified from the 144 h culture was correctly assembled into structures similar to naturally occurring HPV virions. Immunization with the HPV58 L1 efficiently elicited anti-HPV58 neutralizing antibodies and antigen-specific $CD4^+$ and $CD8^+$ T cell proliferations, without the need for adjuvant. Our findings provide a convenient method for obtaining substantial amounts of highly immunogenic HPV58 L1 from *S. cerevisiae*.

Keywords: human papillomavirus, vaccine, *Saccharomyces cerevisiae*, virus-like particle, immunogenicity

Introduction

Human papillomaviruses (HPV) are non-enveloped DNA viruses that infect cutaneous or mucosal epithelial tissues. Most cases of cervical cancer, the second most common cancer in women worldwide, are caused by infection with high-risk HPV types (Walboomers *et al.*, 1999; Bosch *et al.*, 2002). Cervical cancer develops in approximately 500,000 women each year and leads to 250,000 deaths annually (National Cancer Institute, 2007). In addition, it is thought that most women have non-symptomatic HPV infections at some time in their lives (Nyari *et al.*, 2001, 2004). HPV types are divided into two groups: low-risk types such as type 6 and 11 that cause condyloma, and high-risk types such as types 16, 18, 45, 31, 52, and 58, that cause cervical cancer (Clifford *et al.*, 2006). The L1 protein is the major capsid protein of HPV. It can self-assemble into virus-like particles (VLPs), composed of 72 capsomeres (360 L1 proteins) whose structure is similar to that of naturally occurring HPV virions (Conway and Meyers, 2009). The HPV VLP is a major component of prophylactic vaccines for preventing HPV infection, because it displays neutralizing epitopes on its surface (Bishop *et al.*, 2007). Currently, there are two kinds of virus-like particle (VLP)-based prophylactic vaccines. One is Gardasil® (Merck) that uses the *Saccharomyces cerevisiae* (*S. cerevisiae*) expression system, and the other, Cervarix™ (GlaxoSmith-Kline) that uses an insect cell expression system (Madrid-Marina *et al.*, 2009). Gardasil® contains HPV VLPs of types 16, 18, 6, and 11 while Cervarix™ contains HPV VLPs of types 16 and 18. Therefore, these vaccines have HPV VLPs of types 16 and 18 in common (Garland and Smith, 2010).

HPV types 16 (HPV16) and 18 (HPV18) account for about 70% of cervical cancers worldwide (Clifford *et al.*, 2006). However, the geographical distribution of HPV types is quite variable (Clifford *et al.*, 2006). HPV58 is common not only in cervical cancers but also in high grade squamous intraepithelial lesions (HSIL) throughout Asia, Central America and South America (Chan *et al.*, 1999; Hara *et al.*, 2004; Bao *et al.*, 2008; Zhang *et al.*, 2010). Indeed, it is the second and third most common HPV type in Asian cases of cervical cancer and HSIL, respectively (Clifford *et al.*, 2006).

Neutralizing epitopes on the surface of HPV VLPs are highly type-specific because the amino acid sequences in the interior of the VLPs are conserved, whereas those on the exterior are type-specific (Bishop *et al.*, 2007). Although Kemp *et al.* (2011) suggest that vaccination with HPV16 and HPV18 VLPs can elicit cross-neutralizing antibodies to HPV31 and 45, respectively, the majority of the antibodies elicited by the two VLPs are type-specific. Vaccination with HPV16 and 18 VLPs does not elicit cross-neutralizing antibodies against HPV58 (Kemp *et al.*, 2011). Currently, a clinical study indicated that there is no cross-protective immunity for HPV58 in recipients treated with Cervarix™: the vaccine efficacies for HPV31 and 45 for 6-month persistent infection are 77 and 79%, respectively, while that for HPV58 is essen-

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tially 0% (Wheeler *et al*., 2012). Therefore, the development of a new generation vaccine containing HPV58 VLP is a high priority.

To date, HPV58 VLPs have been produced and purified from *Spodoptera frugiperda* cells (insect cell expression system) and *Pichia pastoris* (yeast expression system) (Zhang *et al*., 2010; Jiang *et al*., 2011). However, there has been no successful production and purification of HPV58 VLP from *S. cerevisiae.* Moreover, there has been no study to characterize the immunogenicity of HPV58 VLP from *S. cerevisiae*. In this report we produced HPV58 L1 protein from *S. cerevisiae* and show for the first time that the HPV58 L1s recovered are immunogenic. Our results highlight the factors that need to be considered in order to obtain substantial amounts of highly immunogenic HPV58 VLP and suggest a novel protocol for efficient production of HPV58 VLP.

Materials and Methods

Plasmid construction and yeast transformation

The HPV58 L1 gene was amplified from HPV58 DNA (Matsukura and Sugase, 1990) kindly provided by Dr. Jong Sup Park (College of Medicine, Catholic University of Korea, Seoul, South Korea). The PCR primers designed to amplify the open reading frame (ORF, 1497 bp) of the HPV58 L1 gene were as follows: sense primer, 5′-AAG CTT ATG TTC GTG TGG CGG CCT AGT-3′; antisense primer, 5′-GTC GAC TTA TTT TTT AACCTT TTT GCG-3′. The amplified HPV58 L1 sequence was ligated into the pGEM-T-easy vector and subsequently inserted between the *Hin*dIII and *Sal*I sites of YEGα-MCS to yield YEGα-MCS-HPV58 L1. The YEGα-MCS vector was kindly provided by Prof. Hyun Ah Kang (Chung-Ang University, Seoul, South Korea). We confirmed that the HPV58 L1 gene sequence was identical to that of HM639322.1 in Gene Bank. A detailed plasmid map is presented in Supplementary data Fig. S1. *S. cerevisiae* Y2805 was transformed with YEGα-MCS-HPV58 L1, and transformants were selected on plates containing SD-ura (a synthetic complete medium without uracil). Fifty single colonies were cultured in separate 5 ml aliquots of SD-ura broth for 48 h. Nine cultures with high growth rates were selected, and the levels of production of L1 protein by these isolates were analyzed.

Cell culture

To express HPV58 L1 protein from the GAL10 promoter, the transformants were cultured in YPDG medium containing 1% yeast extract (Duchefa, Netherlands), 2% peptone (Duchefa), 8% glucose (Duchefa), and 1% galactose (Duchefa). The nine candidate cultures were inoculated into 30 or 150 ml of YPDG medium and grown in flasks at 30°C for the indicated times (see Figure Legends) with shaking at 230 rpm. Cell densities were measured at OD 600 nm.

SDS-PAGE and Western blotting

SDS-PAGE was performed according to the method of Laemmli (1970). To determine the volumetric yield of HPV58 L1 by Western blotting, the cells in 1 ml samples of the cultures were pelleted by centrifugation at 10,000×g and disrupted with glass beads in 0.2 ml of breakage buffer (Biospec Product, USA). The lysates were diluted 1:50 with distilled water (DW), mixed with Laemmli sample buffer and boiled for 12 min at 77°C. The band corresponding to L1 protein was detected using rabbit anti-HPV16 L1 polyclonal antibody (Pab) together with anti-rabbit IgG (Pierece, USA) (Kim *et al*., 2010a). The rabbit anti-HPV16 L1 Pab was kindly provided by Dr. J.T. Schiller (NIH, USA). It is known that amino acid sequences of L1 proteins are considerably conserved among certain genital HPV types (Chan *et al*., 1995). Therefore, the anti-HPV16 L1 Pab can be used to detect HPV58 L1 protein for Western blotting (Kim *et al*., 2011). L1 band intensities were determined with NIH open source software Image J, and calculated as described previously (http://rsbweb.nih.gov/ij/).

Purification of HPV58 L1 protein

To purify the HPV58 L1 protein, cells were cultured in 150 ml of YPDG medium for 48 or 144 h, harvested, washed with phosphate-buffered saline (PBS) and mixed with 7 ml of breakage buffer (20 mM sodium phosphate, pH 7.2, 100 mM NaCl, 1.7 mM EDTA, 0.01% Tween 80) and 7 g of glass beads (Biospec Product, USA) (Kim *et al*., 2010a). To break open the cells they were vortexed for 2 min, then rested for 2 min on ice, and this procedure was repeated ten times. The cell debris was then removed by centrifugation at 12,000×g, and supernatant proteins were precipitated by 40% saturated ammonium sulfate (the condition of the precipitate is described in Supplementary data Fig. S2). The precipitate was suspended in 0.9 ml of PBS containing 0.01% Tween 80 and the NaCl concentration was adjusted to 0.8 M with 5 M NaCl. The suspension was stored for 16 h at 4°C, and dialyzed against PBS+0.01% Tween 80 for 4 h. The protein concentration was adjusted to 5 mg/ml with buffer (10 mM sodium phosphate pH 7.2, 150 mM NaCl+0.01% Tween 80), and the suspension was kept at room temperature (RT) for 12 h (removal of precipitated contaminants step, Supplementary data Fig. S2). After removing precipitated protein, the clarified sample was dialyzed against binding buffer (2.68 mM KCl, 1.47 mM KH_2PO_4, 8.1 mM Na_2HPO_4, 0.48 M NaCl, pH 7.0+0.01% Tween 80) prior to cation-exchange chromatography. A column packed with four ml of P-11 phosphocellulose resin (1.5×3.0 cm, P-11 resin, Whatman, UK) was equilibrated with binding buffer, and the dialyzed sample (column loading sample, Supplementary data Fig. S2) was loaded onto the column. The column was washed with seven column volumes of binding buffer, and the HPV58 L1 protein was eluted with buffer containing 0.6, 0.7, 0.8, 0.9, and 1 M NaCl. The L1 protein was collected and concentrated with an Amicon Ultra (Millipore, USA). The amount of L1 protein recovered was determined with a protein assay kit (Bio-Rad, USA).

Transmission electron microscopy (TEM)

Purified HPV58 L1 was dialyzed against PBS+0.2 M NaCl+0.01% Tween 80 (final NaCl conc. 0.33 M), absorbed onto carbon-coated grids and negatively stained with 2% phosphotungstic acid (Kim *et al*., 2010b). Electron microscopy

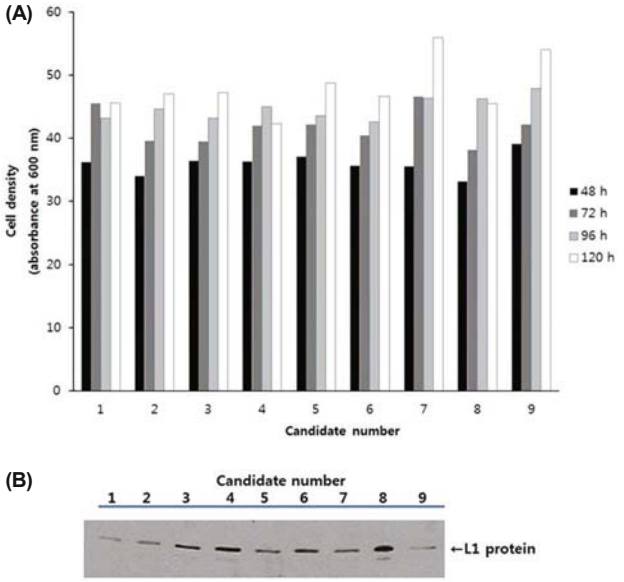

Fig. 1. Growth of HPV58 L1 transformants and yields of L1 protein. Nine independent HPV58 L1 transformants were cultured at 30°C in 30 ml YPDG medium with shaking at 230 rpm. (A) shows the growth of the transformants over 120 h of culture, and (B) their volumetric yields at 120 h. (B) the volumetric yield of L1 protein was determined by Western blotting. See 'Materials and Methods' for further details.

was performed on a TEM200CX at a final magnification of 150,000×.

Ethics

Six-week-old female BALB/c mice were purchased from Orient Bio (Orient Bio, Korea) and acclimatized for 1 week prior to immunization. All animal experiments were performed in accordance with National Research Council Guidelines for the Care and Use of Laboratory Animals and with the Guidelines for Animal Experiments of Chung-Ang University, and were approved by the University Committee for Animal Experiments (approval no. 11-0028).

Immunization of mice

Female mice were divided into three groups: (1) given PBS + aluminum hydroxide (alum), (alum, 200 μg/dose, Sigma, USA) (Sun *et al*., 2008). (2) given 10 μg of HPV58 L1 combined with alum (alum, 200 μg/dose). (3) given 10 μg of HPV58 L1 only. The three groups were subcutaneously immunized three times at two-week intervals. The immunization doses were based on a previous report (Thones *et al*., 2008). Ten days after the final boost, blood was collected by tail vein puncture, and splenocytes were obtained from each group for flow cytometric analysis.

Titration of IgG HPV58 L1 IgG

Titers of anti-HPV58 L1 IgG in the mouse sera were determined by indirect enzyme-linked immunosorbent assay (ELISA) (Woo *et al*., 2007). 96-well ELISA plates were coated with 100 ng of purified HPV58 L1 per well at 4°C overnight and blocked with 2% BSA in PBST. The mouse sera were diluted three-fold, added to the wells and incubated for 1 h at 37°C. The anti-HPV58 L1 IgG bound to the coated HPV58 L1 was detected with HRP-conjugated goat anti-mouse IgG. Color reactions were developed with o-phenylenediamine (Sigma) and measured at 492 nm. End-point titers were taken as twice the OD of the control serum (Kim *et al*., 2007).

Pseudovirus-based neutralizing assay

To investigate the neutralizing ability of anti-HPV58 L1 mouse sera, we used a pseudovirus (PsV)-based neutralizing assay (Pastrana *et al*., 2004). The plasmids p58sheLL (containing both the HPV58 L1 and L2 genes) and pYSEAP (reporter plasmid) were kindly provided by Dr. J.T. Schiller (NIH, USA). For the neutralizing assay, 293TT cells were seeded in 96-well tissue culture plates at 3×10^4 cells/well and incubated for 4 h at 37°C. Optiprep density gradient-purified PsV stock was incubated with serial 3-fold dilutions of the sera at 4°C for 1 h. Thereafter, the 293TT cells were incubated with the PsV-serum mixtures for 72 h at 37°C and the secreted alkaline phosphate (SEAP) activity of the culture supernatants was measured with a colorimetric SEAP

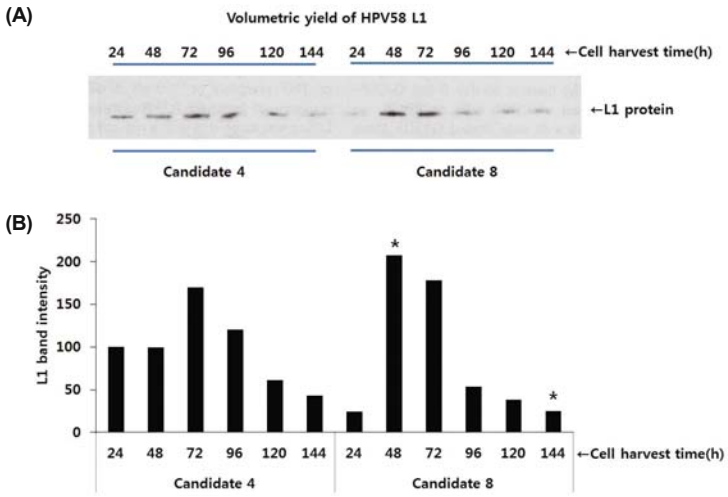

Fig. 2. Volumetric yields of HPV58 L1s during growth. (A) candidate 4 and 8, which gave the highest levels of expression of HPV58 L1 (Fig. 1), were cultured for 24, 48, 72, 96, 120, and 144 h, and their volumetric yields at these times were measured by Western blotting (see 'Materials and Methods'). (B) shows the intensities of the L1 bands in (A). The intensity of the L1 band of candidate 4 at 24 h was set at 100%. Asterisks indicate the samples from which L1 was subsequently purified: the purification results are presented in Figs. 3 and 4.

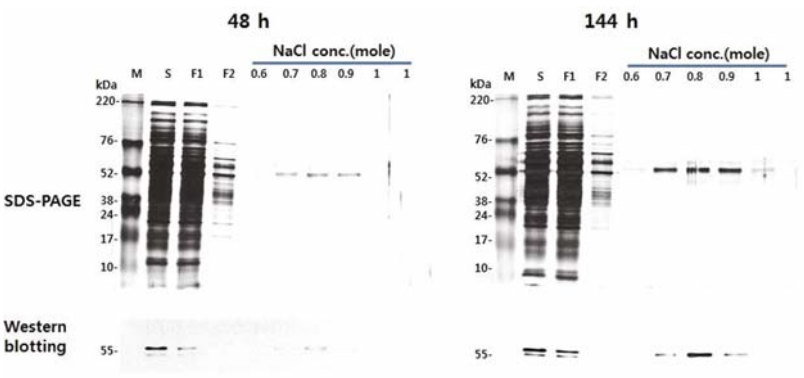

Fig. 3. Cation-exchange chromatography of 48 and 144 h cultures. This shows the results of cation-exchange chromatography of the protein produced by candidate 8 at 48 h and 144 h (asterisks in Fig. 2B). The purifications were performed as described in 'Materials and Methods'. M, S, F1, and F2 refer to molecular weight marker, loading sample, flow-through-1 and flow-through-2, respectively. To elute L1 protein, buffers containing 0.6, 0.7, 0.8, 0.9, and 1 M NaCl (4 ml each) were passed successively through the column. The protein bands on SDS-PAGE gels were visualized by silver staining (upper panel), and L1 protein was detected by Western blotting (bottom panel).

assay. Neutralizing antibody titers were determined as the reciprocal of the highest dilution that caused at least a 50% reduction in SEAP activity when compared to controls treated with PsV alone.

CFSE-based splenocyte proliferation assay

Splenocytes from each mouse group were labeled with carboxyfluorescein succinimidyl ester (CFSE) using a CellTrace^{TM} CFSE cell proliferation kit (Invitrogen, USA). The CFSE-labeled splenocytes were cultured in 24-well, flat-bottom, cell culture plates (5×10^6 cells/well) for 4 days with 10 μg/ml of purified HPV58 L1. They were harvested and stained with anti-mouse CD4 allophycocyanin (APC) antibody (eBioscience, USA) or CD8 peridinin chlorophyll protein complex with cyanin-5.5 (PerCP-Cy5.5, eBioscience). After washing twice with the staining buffer (1% FBS in PBS) the stained cells were analyzed with a FACSAria flow cytometer (BD Bioscience, USA). Ten thousand events were acquired gated on live lymphocytes, and $CD4^+$ or $CD8^+$ cells were gated subsequently to score proliferating $CFSE^{low}$ cells.

Statistical analysis

The statistical significance of differences between groups was determined using two-tailed Student's t-tests. $P<0.05$ was considered a significant difference.

Results

Productivities of HPV58 L1 transformants

To obtain an *S. cerevisiae* strain producing high levels of HPV58 L1 protein, we selected transformants with high growth rates (see 'Materials and Methods'), and measured their volumetric yields of L1 protein. To compare their volumetric yields of L1 protein, extracts of equal volumes of the cells were analyzed by Western blotting. As shown in Fig. 1A, candidates 7 and 9 had the highest growth rates but their yields of HPV58 L1 were not high. We therefore selected candidates 4 and 8, which had the highest volumetric yields (Fig. 1B). We investigated the time-course of HPV58 L1 production by isolates 4 and 8. As shown in Figs. 2A and 2B, their volumetric yields were highest at 72 and 48 h, respectively.

Final amounts of HPV58 L1s recovered

As shown in Fig. 2, the volumetric yield of candidate 8 was highest at 48 h and lowest at 144 h. To investigate the proportions of functional HPV58 L1s contained in the 48 and 144 h cultures, we purified HPV58 L1s by the one-step chromatographic method (Kim *et al.*, 2010a). As shown in Fig. 3, surprisingly, the amount of L1 protein obtained from the 144 h culture was significantly higher than from the 48 h culture, and this result was highly reproducible. As shown in Fig. 4A and Supplementary data Table S1, the mean amount of L1 protein finally recovered from 144 h cultures was 2.3 times higher than from 48 h cultures. TEM analysis showed that the purified HPV58 L1s from 48 and 144 h cultures were correctly assembled (Fig. 4B). This case

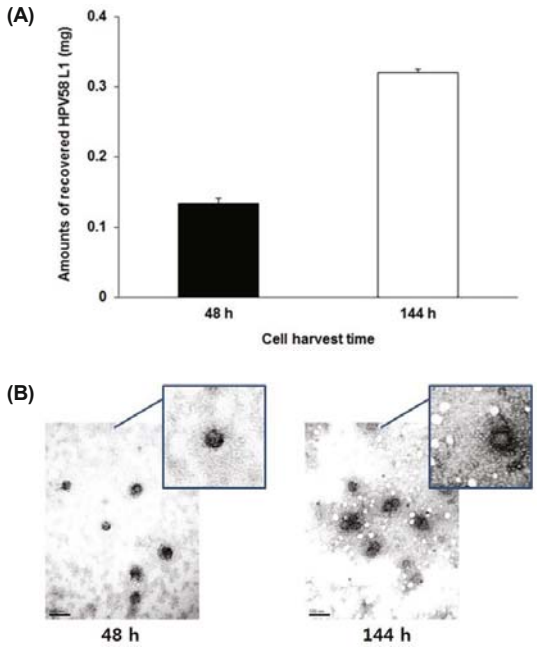

Fig. 4. Comparisons of the amounts and conformations of HPV58 L1 recovered from the 48 and 144 h cultures. To compare amounts of HPV58 L1s finally recovered, the HPV58 L1 in the 48 and 144 h cultures was purified as shown in Fig. 3(A). Detailed purification results are presented in Supplementary data Table S1. Data are means±SD. The results of the TEM analysis are presented in (B). Magnification is 150,000× (bars 100 nm).

was thus unusual in that the final yield of the target protein was not proportional to the upstream yield. We conclude that the longer period of culture is more effective for producing purified HPV58 L1.

Humoral immune responses to HPV58 L1

To investigate the ability to elicit anti-HPV58 L1 IgG and anti-HPV58 neutralizing antibody against HPV58 L1 produced in *S. cerevisiae*, mice were immunized with the HPV58 L1 purified from the 144 h culture above (right panel of Fig. 4B), with and without alum. The detailed immunization protocol is presented in Table 1. Immunization with HPV58 L1 plus alum elicited stronger anti-HPV58 L1 IgG and anti-HPV58 neutralizing antibody responses than HPV58 L1 without alum (Fig. 5). However, the levels of anti-HPV58 L1 IgG and neutralizing antibody titers elicited by HPV58 L1 alone were quite satisfactory. These results indicate that the purified HPV58 VLPs are highly immunogenic and use of alum as an adjuvant is effective to increase the humoral immune responses.

Antigen specific-CD4$^+$ and CD8$^+$ T cell proliferation following immunization with HPV58 L1

To investigate cell-mediated immune responses, mice were immunized three times with HPV58 L1, and splenocytes were re-stimulated with HPV58 L1 (see 'Materials and Methods'). Four days after re-stimulation, proliferating CD4$^+$ and CD8$^+$ T cells were scored (Fig. 6). The proportions of proliferating CD4$^+$ and CD8$^+$ T cells in the HPV58 L1 + alum-immunization group were about 1.3 times higher

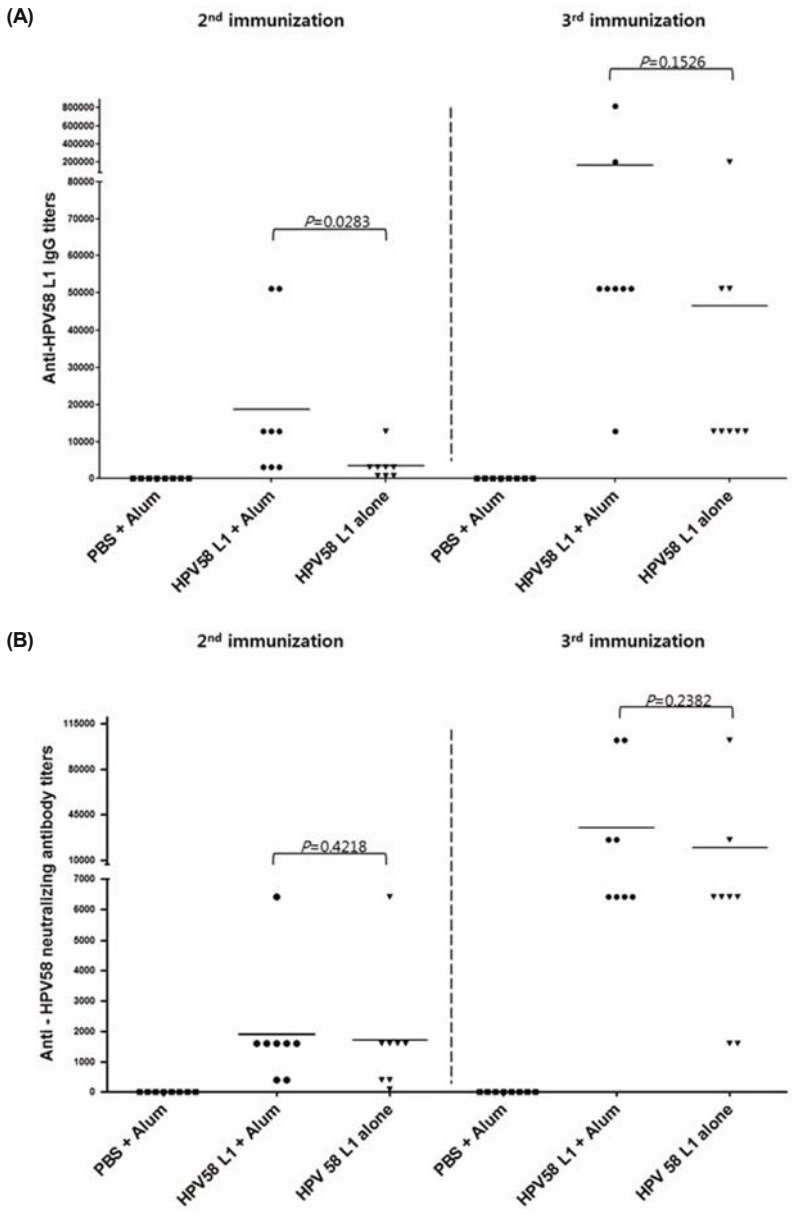

Fig. 5. Humoral immune responses induced by immunization with HPV58 L1. Mice were immunized subcutaneously three times as described in 'Materials and Methods'. The titers of anti-HPV58 L1 IgG and anti-HPV58 neutralizing antibodies obtained are presented in (A) and (B), respectively. For details of immunization and assays see 'Materials and Methods' and Table 1. Titers are represented as dots for the eight individual mice (n=8). Horizontal bars indicate mean titers of anti-HPV16 L1 IgG and neutralizing antibody.

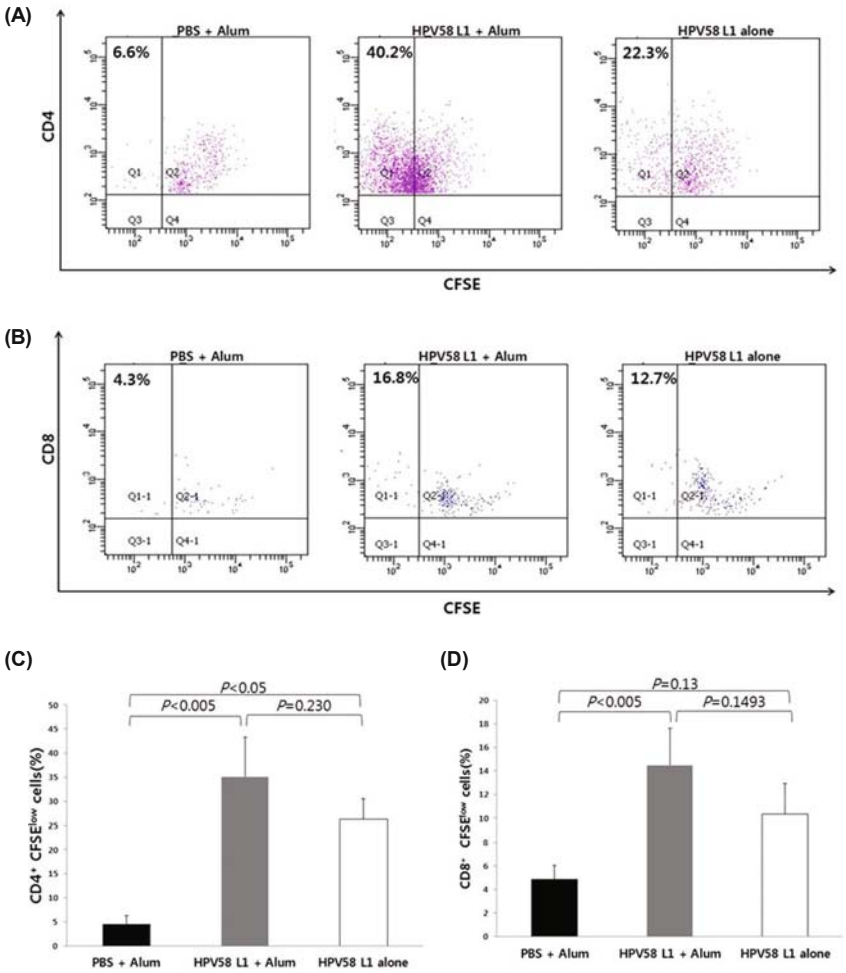

Fig. 6. Antigen-specific lymphoproliferative responses following immunization with HPV58 L1. To measure HPV58 L1-specific $CD4^+$ and $CD8^+$ cell proliferation, mice were immunized subcutaneously three times as described in Table 1. Ten days after the last immunization, mice spleens were isolated and labeled with CFSE. The CFSE-labeled spleen cells were re-stimulated with 10 μg/ml of HPV58 L1 and cultured for 4 days. $CD4^+$ and $CD8^+$ cells were detected using anti-mouse CD4 APC and CD8 PerCP antibodies. (A) and (B) are representative flow cytometry results for six individual mice, showing the proliferation of $CD4^+$ and $CD8^+$ cells. To score proliferating $CD4^+$ and $CD8^+$ cells, $CD4^+$ and $CD8^+$ cells were gated from live lymphocytes, and the upper left segment of each graph was counted. (C) and (D) are means±SEM (n=6).

than those in the HPV58 L1 alone group (Figs. 6C and 6D). Therefore, the use of alum in immunization with HPV58 L1 was helpful in eliciting cell-mediated immune responses. Meanwhile, HPV58 L1 alone induced healthy antigen-specific proliferative responses by the T cells. It is evident therefore that the HPV58 L1 produced in *S. cerevisiae* has sufficient immunogenicity and quality to encourage its use as a prophylactic vaccine.

Discussion

We have produced HPV58 L1 protein in *S. cerevisiae* and confirmed that the HPV58 L1 is correctly assembled and highly immunogenic. Previously, we confirmed that HPV16 and 18 L1 were produced successfully from *S. cerevisiae* cultured in YPDG medium containing 2–4% carbon sources (Kim *et al.*, 2009, 2010a). However, we failed to detect any HPV58 L1 production when the *S. cerevisiae* was grown with 2–4% carbon sources (data not shown). In the present study, therefore, we used a 9% carbon source for producing HPV58 L1 protein, and this significantly increased the production of HPV58 L1 to a level detectable by Western blotting (Fig. 1B). This suggests that the percentage of carbon source supplemented in culture medium is critical for the production HPV58 L1 protein.

Our observation that the yield of L1 protein finally recovered from 144 h cultures was much higher than that from 48 h cultures, even though the volumetric yield of L1 protein at 48 h was higher than that at 144 h (Figs. 2, 3, and 4A), suggests that the HPV58 L1 is more stable in 144 h cells than 48 h cells. The disulfide bonding between L1 proteins is known to be critical in physical stability of the VLP (Buck *et al.*, 2005). In addition, the mature HPV VLPs, whose disulfide bonds are extended, are resistant to proteolysis (Buck *et al.*, 2005). Moreover, it has been shown that the extension of disulfide bonds can be facilitated by exposure to oxidized glutathione *in vitro* (Buck *et al.*, 2005). Later, Conway *et al.* (2009) showed that the structural integrity and infectivity of the HPV capsid differed depending on the period of cell culture. We have confirmed that the intracellular oxidized glutathione levels are significantly different according to the length of the culture period (unpublished data). Therefore, it is assumed that the time of cell harvesting influences the structural integrity and stability of the VLPs. Accordingly, we suggest that the period of cell culture should be considered to be a significant parameter in the production of HPV58 L1.

We confirmed that the purified HPV58 VLPs have superior immunogenicity and use of alum as an adjuvant effectively increases both humoral and cell-mediated immune responses. The assembled structure of the HPV L1 protein is known to be critical for its immunogenicity: the disassembled form of HPV16 L1 elicited significantly lower levels of anti-HPV16 IgG and neutralizing antibody in mice (Thones *et al*., 2008). In addition, the use of alum as adjuvant along with disassembled HPV16 L1 failed to increase antibody titers (Thones *et al*., 2008). Previously, we developed a purification method for HPV16 and 18 L1 using cation-exchange chromatography (Kim *et al*., 2009, 2010a). The HPV VLPs purified by that method showed superior immunogenicity (Kim *et al*., 2007; Woo *et al*., 2007). In cation-exchange chromatography with phosphocellulose resin, the disulfide bonds between L1 proteins are important for binding of assembled L1 protein to the resin; monomeric L1 protein does not bind (unpublished data). Therefore, we think that the cation-exchange chromatography plays a role in selecting forms of L1 protein useful as a vaccine.

Until recently most methods for purifying HPV L1 produced in yeast have required several chromatographic steps (Park *et al*., 2008; Kim *et al*., 2012). In the present case we purified 0.32 mg of HPV58 L1 from 150 ml of *S. cerevisiae* culture by a one-step chromatography method. This amount of L1 protein is sufficient for 60 ELISA plates for antibody titration or 16 doses of vaccine for humans, as well as for most experiments associated with HPV58.

In the production of recombinant proteins, the prokaryotic expression system is known to be simple and convenient. However, the main hurdle for VLP production using the prokaryotic expression system is obtaining soluble and full-length protein (Pattenden *et al*., 2005). In addition, having bacterial toxin contaminating the final VLP product has hindered its use as a commercial vaccine (Schadlich *et al*., 2009). These disadvantages of the prokaryotic expression system can make the prokaryote-derived VLPs potentially more expensive than those produced in a eukaryotic expression system. The insect cell system can efficiently produce VLPs, which have a high structural consistency, but it has higher production costs than the yeast or prokaryotic expression systems (Pattenden *et al*., 2005; Vicente *et al*., 2011). The VLP produced from *S. cerevisiae* is known to have superior structural integrity, while its production cost is lower than insect cell or mammalian cell expression systems (Kim *et al*., 2012). In addition, the safety and efficacy of biopharmaceuticals produced from *S. cerevisiae* are better defined than other yeast expression systems such as *Pichia pastoris* or *Hansenula polymorpha* (Stockmann *et al*., 2009). Therefore, we expect that refinement of our procedure for producing HPV58 VLP should permit the rapid development of a commercial prophylactic vaccine, and aid in preventing cervical cancer.

Acknowledgements

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