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Short communication

Evaluation of the enhancing ability of three adjuvants for DNA vaccination using the porcine circovirus type 2 *ORF2* (capsid) gene in mice

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ABSTRACT

Molecular adjuvants were used to augment the amplitude of the immune response in many studies recently. Ubiquitin (ub), the peptide binding truncated C-terminal portion of heat shock protein 70 (hsp70c) and interleukin-2 (IL-2) are widely investigated adjuvants which have been proved to be efficient. In our study, we compared the enhancing ability of these three adjuvants based on DNA vaccination using the porcine circovirus type 2 ORF2 (capsid) gene in mice. The results of lymphocyte proliferation assay, flow cytometric analysis (FCM), antibody titer and cytokine production showed that ub conjugated plasmid induced a stronger Th1 type cellular immune response and an observably higher level of Cap-specific serum immunoglobulin G antibody compared with hsp70c or IL-2 conjugated plasmids during the period of post-immunization. Meanwhile, the ub conjugation vaccinated group elicited stronger specific immunity against PCV2 challenge than the others during most of the time of post-challenge. Thus, these data indicate that ub is a superior adjuvant for a PCV2 DNA vaccination than the hsp70c and IL-2 molecules.

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Molecularly encoded adjuvants were widely used in DNA vaccine due to their ability to enhance DNA delivery and increase the duration and magnitude of plasmid DNA expression. GroEL, one of the heat shock proteins (HSPs), has been fused with a plasmid which encodes endothelin A receptor (ETAR) elicited remarkable and specific antibody responses to native ETAR (Fujimoto et al., 2012). IL-2 was reported that it could enhance antigen-specific T cell-dependent immunity and antibody-mediated immunity responses (Chow et al., 1997; Kim et al., 2001; Nobiron et al., 2001; Bu et al., 2003; Karina et al., 2010). The C-terminal peptide-binding portion of hsp70c, has been shown to be responsible for stimulating Th1-polarising cytokines, C–C chemokines and adjuvant function (Wang et al., 2002). Ubiquitin (ub) has been used as a helpful adjuvant to enhance the immune response in *Tuberculosis* DNA vaccination (Delogu et al., 2000) and influenza virus DNA vaccine (Fu et al., 1998). Our former researches also concluded that Ubiquitin (ub) and the peptide binding truncated C-terminal portion of heat shock protein 70 (hsp70c) could improve both the cellular and humoral immune responses of DNA vaccination using the porcine circovirus type 2 ORF2 (Cap) gene in mice (Fang et al., 2011, 2012).

Though ub, hsp70c and IL-2 have been proved to be efficient as adjuvants in different researches (Delogu et al., 2000; Li et al., 2006; Moore et al., 2002), they have never been compared with each other based on the same DNA vaccination. In the present study, we made comparations between these three adjuvants based on PCV2 DNA vaccination by means of surveilling the changes of antibodies, SI, CD4+ and CD8+ T cells, IFN-γ and IL-2, and the viral load.

Since the recombinant plasmids, pCA-Cap, pCA-TCHc and pCA-UBc were available (Fang et al., 2011, 2012), we only generate the coexpression plasmid pCA-IL-2c. PCA-IL-2c, a 471 bp DNA fragment encoding the swine-origin IL-2 gene, was amplified by PCR using primer pairs FW2/RV2. The PCR product digested with *Sac*I and *Xho* I was subcloned into the expression vector pCAGGS to construct the recombinant expression plasmid pCA-IL-2. The recombinant expression plasmid pCA-IL-2c was generated by linking pCA-IL-2 with pCA-*Cap* that had been digested with *Sac*I and *Xho* I. Finally, the plasmids were purified using an EndoFree Plasmid Giga kit column (Qiagen). Primers used for PCR amplification were listed in Table 1.

Five groups (25 per group) of 8-week-old female BALB/c mice were immunized three times at 3 week intervals with 100 μg vector, pCA-Cap, pCA-TCHc, pCA-UBc or pCA-IL-2c respectively (Fang et al., 2011). The study was approved by the Harbin Veterinary

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Table 1
Oligonucleotide primers.

Gene	Primers	Sequences of primers (5′ → 3′)	Length (bp)	Annealing temperature (°C)
PCV2 *ORF2*	FW1	AA*TCTCGAGCGCCACCATG*ACGTATCCAAGG	717	56
	RV1	ATT*AGATCT*TTATTCATTAAGGGTTAAGTG		
IL-2	FW2	CCTGAGCTCGCCACCATGTATAAGATG	471	54
	RV2	CCCCTCGAGAGTCAGTGTTGAGTA		
ub-linker	FW3	gga *gagctc*gccaccatgcagatcttcgtg	270	56
	RV3	gccgcgccgctgccgccgccgctgccgccgccgctcctcccctcaagcg		
Linker-ORF2	FW4	ggcggcggcggcagcggcggcggcagcggcggcggcATGACGTATCCAAGG	790 bp	56
	RV4	*ctcgag* TTATTCATTAAGGGTTAAGTGGGGGG		
U-ORF2	FW5	gga *gagctc* gccaccatgcagatcttcgtg	1030bp	56
	RV5	*ctcgag* TTATTCATTAAGGGTTAAGTGGGGGG		
TCR2	TCF1	ggaga*gctc*gccaccatggatgcaatgaagaggcctcgtgtgctgctgctgtggagcagtcttcgtttcgAATGGCATCTTCAAC	669 bp	51 °C
	TCR2	ggggca*tgc*TTATTCATTAAGGGTTAAGTGGGGG		
M. tuberculosis hsp70c	LHcF2	g*cggcatg*cgccaccgaggtgaaagacgttc	771 bp	68 °C
	LHcR1	ata*agatct*atcagccgagccggg		

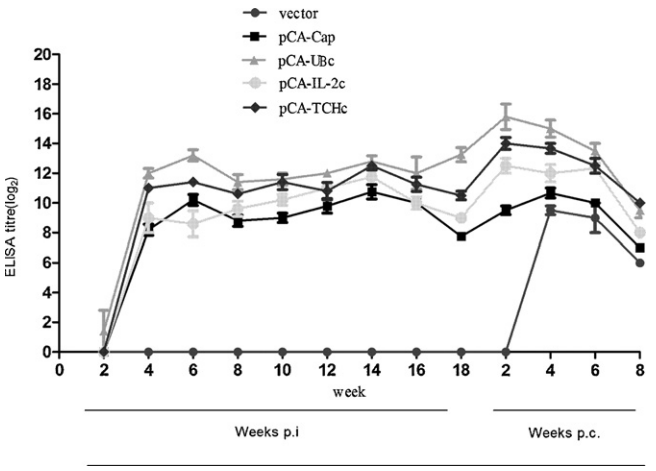

Fig. 1. Kinetics of total immunoglobulin G(IgG) production against porcine circovirus type 2 (PCV2) capsid (Cap) protein at various times post-immunization (p.i.) and post-challenge (p.c.), following with DNA immunization. The results are shown as means ± SD ($n = 5$).

Research Institute Animal Experiment Ethics Committee (approval number SYXK Hei 2006-032). Serum samples were collected every 2 weeks after immunization until the end of the experiment for Enzyme-Linked Immunosorbent Assay (ELISA) analysis. Five mice from each group were sacrificed and tissues were collected 6 weeks after the final injection. Samples are collected for lymphocyte proliferation assays, FCM for CD4+ and CD8+ T cells, and cytokine assays by ELISA for IFN-γ and IL-2 as described before (Fang et al., 2011).

At 18 weeks post-immunization, the remaining 20 mice from each group were challenged intra-peritoneally with 0.5 mL inoculum containing $10^{6.5}$ 50% tissue culture infective doses/mL PCV2 strain 871 (EU420015). Five mice from each group were euthanized at 2, 4, 6 and 8 weeks post-challenge, and whole blood was collected for quantification of viral load by real-time PCR for PCV2 Rep.

As shown in Fig. 1, the titers for total IgG against PCV2 Cap protein in the pCA-IL-2c, pCA-UBc, pCA-TCHc and pCA-Cap groups were higher than the vector groups all through the experiment. Mice immunized with pCA-UBc had higher titers of IgG compared to the titers from the pCA-IL-2c or the pCA-TCHc group during the period of post-immunization (p.i.), indicating ub is a superior molecular adjuvant than hsp70c and IL-2 in inducing higher titers of IgG.

In general, a balance between Th2 and Th1 types of cellular responses is required for protection against viruses (Stevens et al., 1988). Inducing a strong Cap-specific Th1 immune response is important for protective immunity against PCV2 infection (Shen et al., 2008). Th1 cells which could induce activation of macrophages and the production of IgG2a, produce IFN-γ and IL-2 (Abbas et al., 1996; Mosmann and Coffman, 1989). Therefore, IgG isotype profiles, IL-2 and IFN-γ were investigated in this study to assess the influences of the three adjuvants for the cell-mediated immune responses against PCV2.

In Fig. 2, we could observe titers of IgG2a in all groups were significantly higher than that of IgG1 and titers of IgG2a of pCA-UBc immunized group increased sharply at 6 week post-challenge (Fig. 2f). Fig. 3 showed that the expressions of IFN- and IL-2 was higher in splenocytes of mice immunized with the ORF2 gene fused to IL-2, ub and the hsp70 C-terminus than in mice immunized with the ORF2 gene alone. The group of mice immunized with pCA-UBc generated significantly higher ($P < 0.05$) levels of IFN-γ and IL-2 than the other groups. In the assays of T cell proliferation and FCM, the highest level of upregulation of CD4+ and CD8+ T cells and SI

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