Antigen delivery to antigen presenting cells for adaptive immune response by self-assembled anionic polysaccharide nanogel vaccines

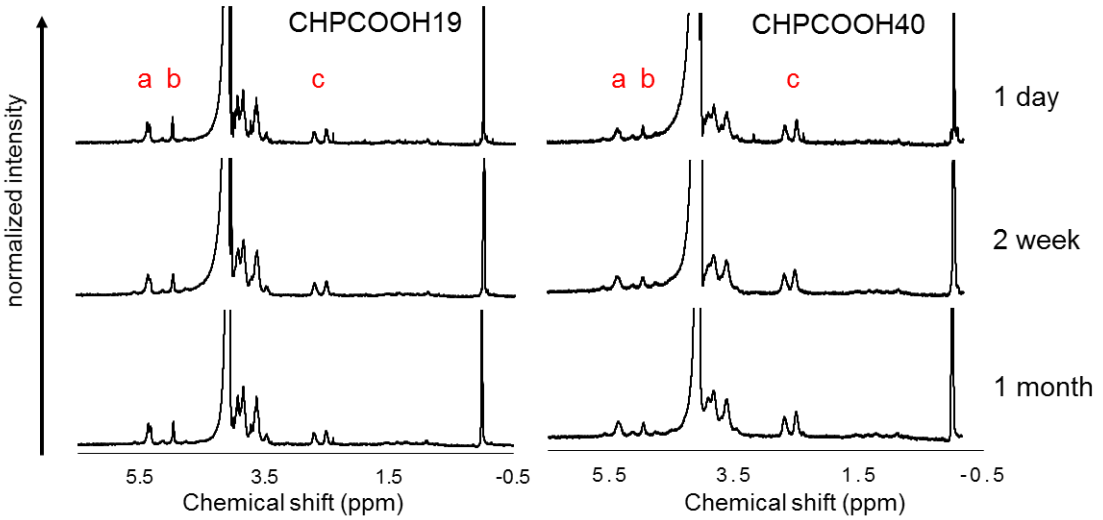
Risako Miura, Shin-ichi Sawada, Sada-atsu Mukai, Yoshihiro Sasaki and Kazunari Akiyoshi*

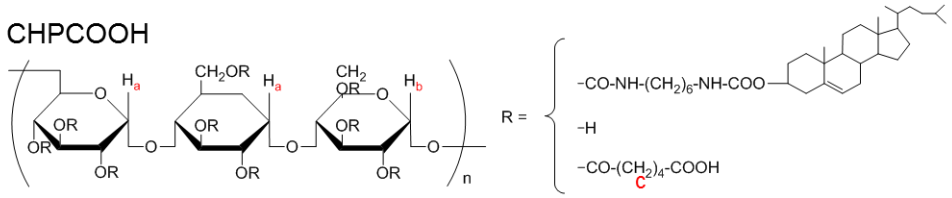
Department of Polymer Chemistry, Graduate School of Engineering, Kyoto University, Japan

Corresponding author: Kazunari Akiyoshi
Tel: +81-75-383-2594
E-mail: akiyoshi@bio.polym.kyoto-u.ac.jp

Materials

CHP was purchased from NOF Corporation (Tokyo, Japan). Phosphate-buffered saline, RPMI1640, DMEM, fetal bovine serum and antibiotic-antimycotic were purchased from Gibco (Carlsbad, CA, USA). EndoGrade OVA was purchased from Hyglos GmbH (Bernried, Germany). Succinic anhydride was purchased from KANTO Chemical co., Inc. (Tokyo, Japan). Urea, dimethyl sulfoxide and 4-dimethylaminopyridine were purchased from Wako (Osaka, Japan). Slide-A-Lyzer^{TM} Dialysis Cassettes (10 K MWCO, 3 mL) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). DQ Ovalbumin (DQ-OVA) was purchased from Invitrogen (Carlsbad, CA, USA). CpG with phosphorothioate modification was purchased from FASMAC (Kanagawa, Japan). PE anti-mouse H-2K^{b} bound to SIINFEKL antibody (25-D1.16), APC anti-mouse CD8 antibody (53-6.7), PE anti-mouse IFN-γ antibody (XMG1.2), PE anti-mouse CD11c antibody (N418), Pacific Blue anti-mouse CD11b antibody (M1/70), PE/Cy7 anti-mouse F4/80 antibody (BM8), PE/Cy7 anti-mouse B220 antibody (RA3-6B2), PE/Cy7 anti-mouse CD103 antibody (2E7), PE/Cy7 anti-mouse CD8 antibody (53-6.7), and the Alexa 488 anti-mouse B220 antibody (RA3-6B2) were purchased from BioLegend (San Diego, CA, USA). The anti-CD207 (Langerin) monoclonal antibody Alexa Fluor 488 (eBioRMUL.2) and CD204 monoclonal antibody PE (M204PA) were purchased from eBioscience (San Diego, CA, USA). GoldiPlug and Cytofix/Cytoperm Kits were purchased from BD Bioscience (San Jose, CA, USA). Bovine serum albumin, 3,3',5,5'-tetramethylbenzidine liquid substrate, polyinosinic acid potassium salt homopolymer and collagenase (type IV) were purchased from Sigma-Aldrich (St. Louis, MO, USA) Fucoidan was purchased from Cayman Chemical Company (Ann Arbor, MI, USA).

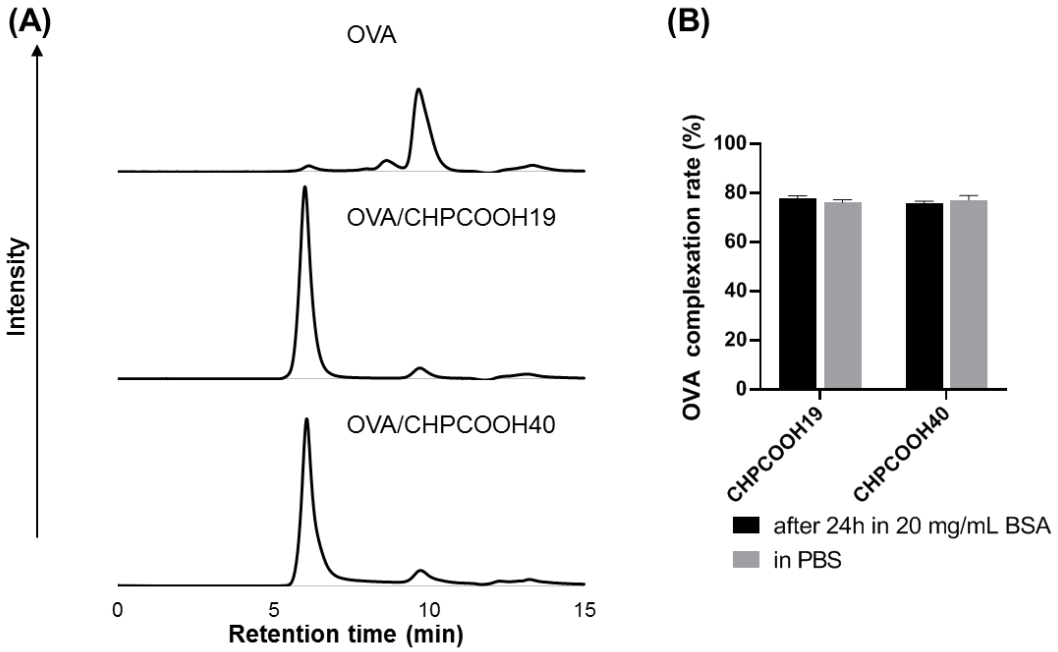




	Integration of peak c	
Time	**CHPCOOH19**	**CHPCOOH40**
1 day	83	150
2 week	85	147
1 month	83	133

peak a+b ≒ 100, as 100 glucose units

Figure S1. ^{1}H-NMR spectra of CHPCOOH19 and 40. The carboxyl group peaks remained unchanged for at least 1 month, which indicated the stability of carboxyl group modification.



	retention time	
peak area	6 min (nanogels)	10 min (OVA alone)
OVA	-	1.9×10^5
CHPCOOH19	3.3×10^5	2.4×10^4
CHPCOOH40	3.1×10^5	3.0×10^4

Figure S2. Complexation and release rate of CHPCOOH nanogel vaccines. **(A)** SEC histogram and **(B)** the complexation rate and release rate of OVA. CHPCOOH nanogel vaccines showed almost the same complexation rate after mixing with 20 mg/mL BSA for 24 h at 37°C.

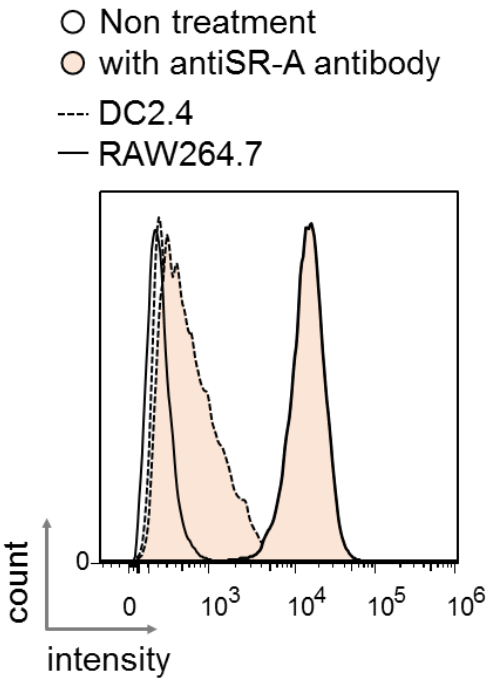


Figure S3. SR-A expression on APCs. DC2.4 cells (dashed line) and RAW264.7 cells (solid line) were immunostained with PE anti SR-A antibody, and the fluorescence intensity was evaluated by flow cytometry. Both cell populations expressed SR-A, and RAW264.7 cells showed higher expression on the cell membrane.

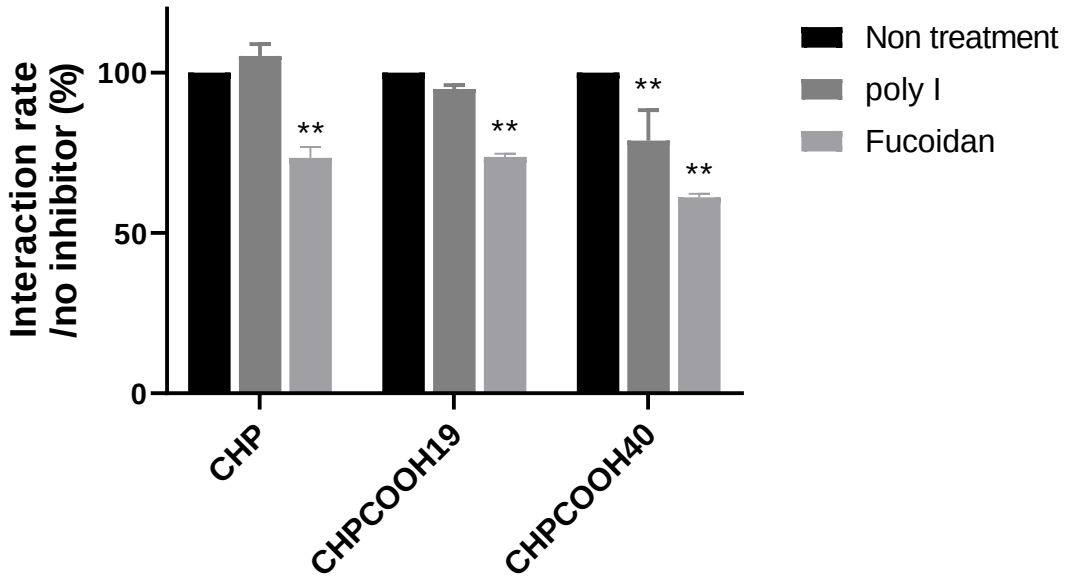


Figure S4. Binding inhibition assay of nanogel vaccines with RAW264.7 cells. RAW264.7 cells were pre-treated with 100 μg/mL polyinosinic acid (poly I) or 5 mg/mL fucoidan (ligands of SR-A) and then treated with nanogel vaccines. The interaction of CHPCOOH40 with RAW264.7 cells was more inhibited than that of CHPCOOH19 nanogel vaccines, which indicated that CHPCOOH40 nanogel vaccine interacted via SR-A more strongly than CHPCOOH19. (**: $p < 0.01$)

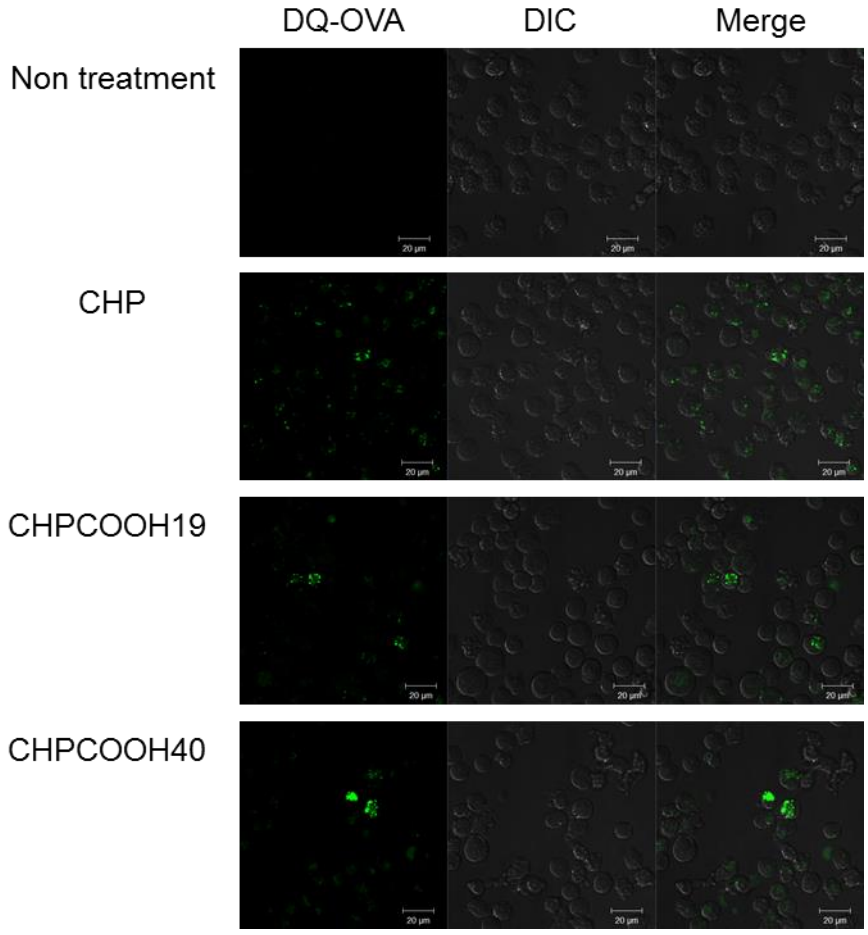


Figure S5. CLMS observation of the interaction of DQ-OVA loaded nanogel vaccines and DC2.4 cells. Strong green fluorescence suggests OVA hydrolysis in the cells (scale bar: 20 μm).

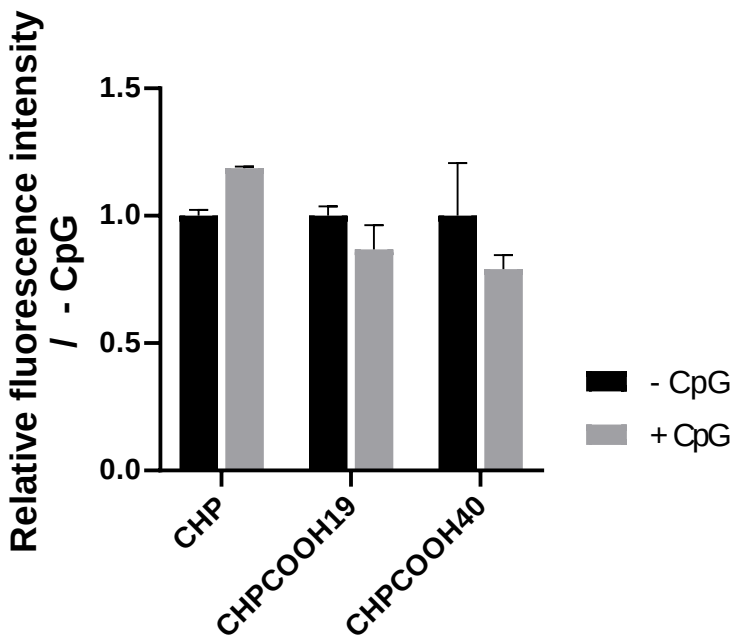


Figure S6. Uptake of OVA-Cy5.5-loaded CHPCOOH nanogels by DC2.4 cells was evaluated by flow cytometry. Both nanogels showed no difference in uptake pattern between with and without CpG DNA cases. ANOVA analysis showed no significant difference within each nanogel group between groups with and without CpG.

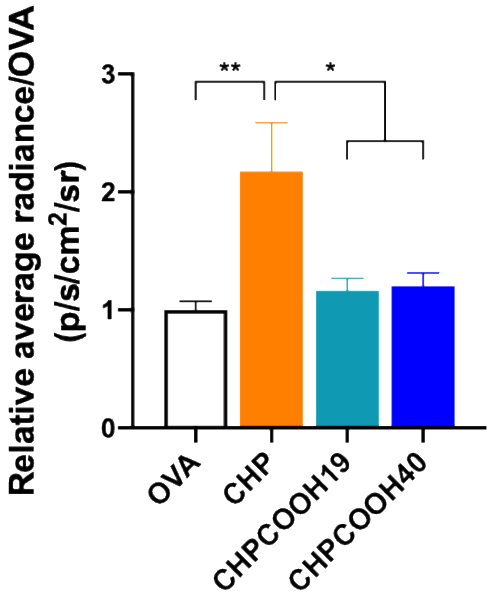


Figure S7. Accumulation of OVA-Cy5.5/nanogels complexes in DLNs at 6 hours after subcutaneous injection, analyzed by IVIS. CHPCOOH nanogels showed less accumulation in DLNs compared to CHP nanogel. (*: $p < 0.05$, **: $p < 0.01$)