Supporting Information

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SI Text

Zwitterionic Glycoconjugates. The zwitterionic PS were obtained as described in ref. 1 by the introduction of a positive charge in the aliphatic chain of the terminal N-acetylneuraminic acid (NeuNAc) residue. The covalent attachment of the carrier protein CRM_{197} or HSA to the zwitterionic PS was performed according to the protocol used for the conjugation of the native CPS (2). The crucial step in the zwitterionization, first, and in the conjugation, in a second time, is the initial oxidation that has to occur only for 10–30% of NeuNAc residues. Therefore, the final glycoconjugate has 10–30% of NeuNAc residues modified with a positive charge, 10–30% of NeuNAc residues implicated in the covalent binding with the carrier protein, and the rest of NeuNAc residues unaltered. ZPS-conjugates were generated by using ZPS from serotypes Ib and V. ZPS-conjugates were purified by gel filtration chromatography on the Sephacryl S-300 HR column. Polysaccharide content of ZPS and ZPS-conjugate preparations was estimated by the colorimetric detection of sialic acid residues with the Svennerholm method (3). The microBCA kit assay (Pierce) was used to estimate the protein content of ZPS-conjugate sample. The polysaccharide/protein ratio for all glycoconjugates used here was measured to be 1:1 (w:w).

Nuclear Magnetic Resonance. Nuclear magnetic resonance (NMR) was used to assess the structural identity of the native PS-conjugate and the chemically derived ZPS-conjugate. NMR spectra were recorded at 25 °C on a Bruker DRX 600 MHz spectrometer by using a 5-mm triple-resonance NMR probe (Bruker). For data acquisition and processing, XWINNMR 2.6 software package (Bruker) was used. NMR samples were prepared by dissolving lyophilized product in 0.75 mL deuterium oxide (D_2O, Aldrich) to a uniform concentration and transferred to 5-mm NMR tubes (Wilmad). 1-D proton NMR spectra were collected by using a standard one-pulse experiment and collecting 32,000 data points over a spectral window of 6,000 Hz. The complete relaxation of all nuclei was assured. The spectrum was Fourier-transformed after applying a 0.2-Hz line broadening function and referenced relative to mono-deuterated water (HDO) at 4.79 ppm.

1. Gallorini S, et al. (2007) Introduction of zwitterionic motifs into bacterial polysaccharides generates TLR2 agonists able to activate APCs. *J Immunol* 179:8208–8215.
2. Wessels MR, et al. (1990) Immunogenicity in animals of a polysaccharide-protein conjugate vaccine against type III group B *Streptococcus*. *J Clin Invest* 86:1428–1433.
3. Svennerholm L (1957) Quantitative estimation of sialic acids. II. A colorimetric resorcinol-hydrochloric acid method. *Biochim Biophys Acta* 24:604–611.

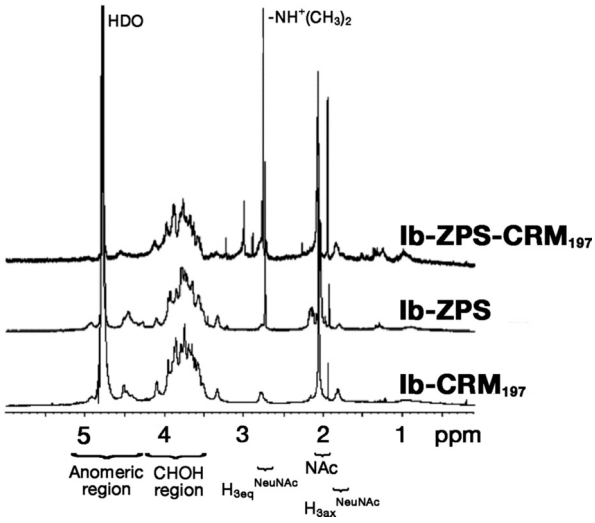

Fig. S1. Zwitterionic polysaccharide conjugation. ^{1}H NMR 600 MHz spectra (spectral window from 0 to 6 ppm) recorded at 25 °C of the Ib-CRM$_{197}$ conjugate (bottom line), Ib-ZPS polysaccharide (central line) and the Ib-ZPS-CRM$_{197}$ conjugate (top line). The peak of the methyl group -NH^{+}(CH$_3$)$_2$ that was chemically introduced in the ZPS before conjugation is present in the glycoconjugate as annotated. Other labels are indicated to facilitate the assignment of the peaks.

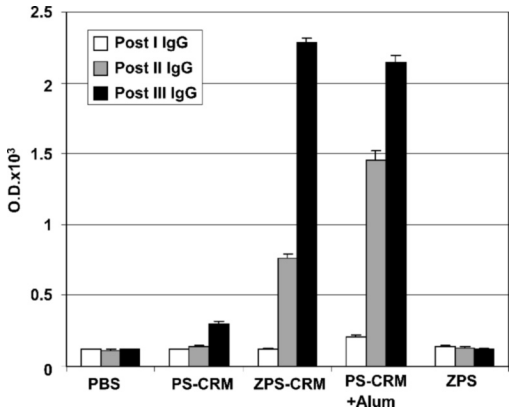

Fig. S2. ZPS accelerate the induction of IgG. Balb/C mice were immunized i.p. with three doses of the PS-conjugates (1 μg) with or without alum (0.4 mg), ZPS-conjugates or ZPS. PBS was used as negative control. Two weeks post first, second, and third dose, sera were analyzed for PS-specific IgG. Results are mean of triplicates + SD. ZPS-CRM and alum-adjuvanted PS-CRM accelerate the induction of IgG compared to PS-CRM alone. Unconjugated ZPS do not induce any PS-specific IgG.

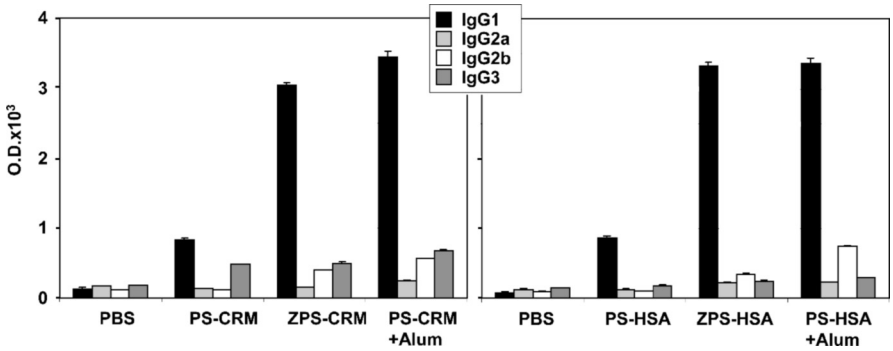

Fig. S3. ZPS-conjugates and alum increase the same PS-specific IgG subclasses. Balb/C mice were immunized i.p. with three doses of the PS-conjugates (1 μg) with or without alum (0.4 mg), ZPS-conjugates (1 μg), and PBS as negative control. Two weeks post third dose, sera were analyzed for PS-specific IgG subclasses. ZPS-conjugates and alum increase the same PS-specific IgG subclasses that are above all IgG1.

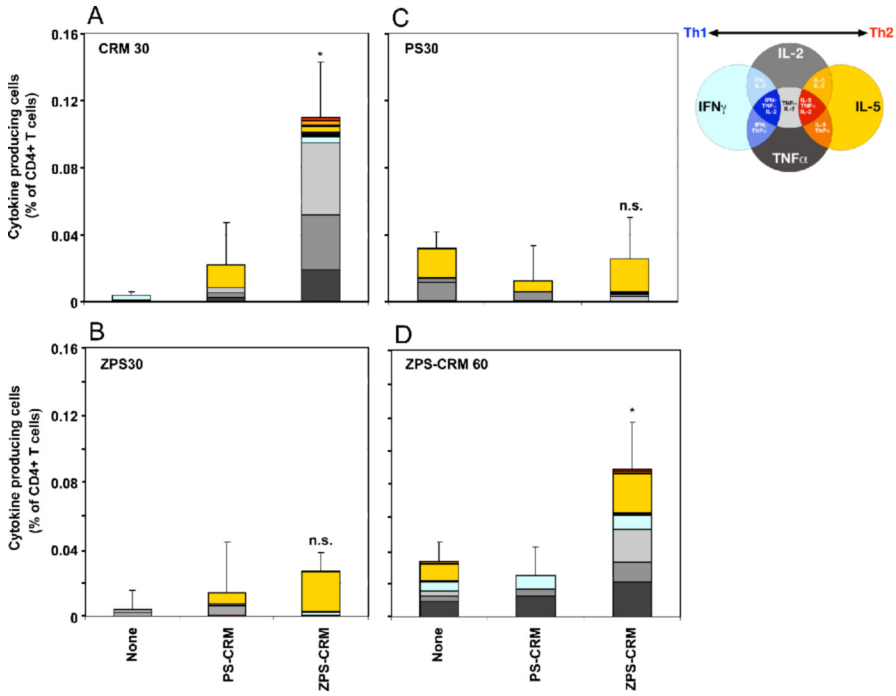

Fig. S4. ZPS do not induce a $CD4^+$ ZPS-specific T-cell recall response. Balb/C mice were immunized i.p. with three doses of the PS-conjugates (1 μg), ZPS-conjugates (1 μg), and PBS as negative control. Two weeks post third dose, spleens were tested for $CD4^+$ T-cell cytokine responses to CRM_{197} (30 μg/mL), to ZPS (30 μg/mL), to PS (30 μg/mL), and to ZPS-CRM (60 μg/mL). ZPS enhance the T-cell cytokine recall response to CRM197 (*A*), but they do not induce a significant T-cell cytokine recall response to ZPS or PS (*B* and *C*). Thus, the increase in $CD4^+$ T-cell response to ZPS-CRM (*D*) is exclusively to the CRM portion because ZPS do not induce a ZPS-specific T-cell response (*B*). Error bars, SD of total percentage of all cytokines of three mice. This experiment was performed two times with similar results. Statistical significance was analyzed by using Student's *t* test. *, $P < 0{,}05$; n.s., not significant compared with PBS and PS-CRM.

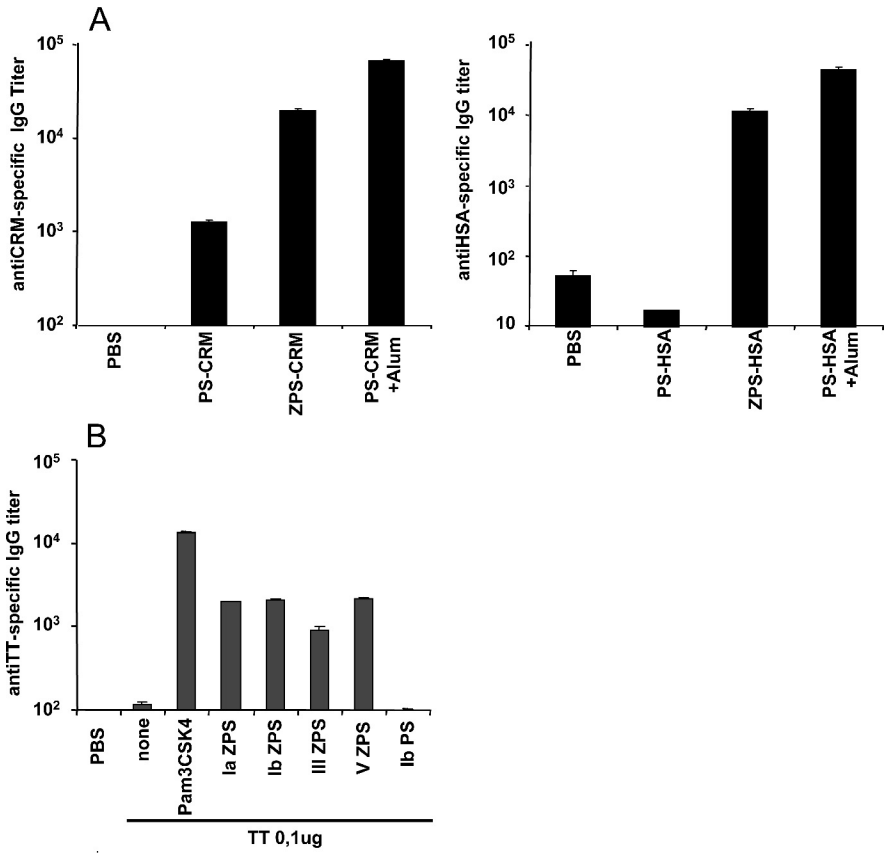

Fig. S5. ZPS enhance responses both to conjugated and unconjugated proteins. (*A*) Balb/C mice were immunized i.p. with three doses of the PS-conjugates (1 μg) with or without alum (0.4 mg), ZPS-conjugates (1 μg), or PBS as negative control. Two weeks post third dose, sera were analyzed for protein (CRM or HSA)-specific IgG titer. (*B*) Balb/C mice were immunized s.c. with tetanus toxoid (0,1 μg) alone or in combination with admixed ZPS serotypes Ia, Ib, III, and V or the native PS serotype Ib. Pam_3CSK_4 was used as positive control and PBS as negative control. Mice received two doses at days 1 and 21, and 2 weeks after the second dose, sera were assessed by ELISA for TT-specific IgG antibody titers. Results are geometric means of triplicates + SD.

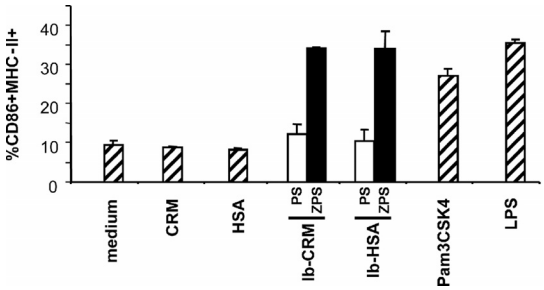

Fig. S6. BM-DCs are activated by ZPS-conjugates. Mouse BM-DCs were incubated for 20 h with ZPS and PS conjugates (10 μg/mL), CRM_{197} and HSA alone (10 μg/mL), and Pam_3CSK_4 or LPS (1 μg/mL). The upregulation of CD86 and MHC class II was evaluated by flow cytometry. Data represent mean + SD of triplicates and are representative of three experiments.

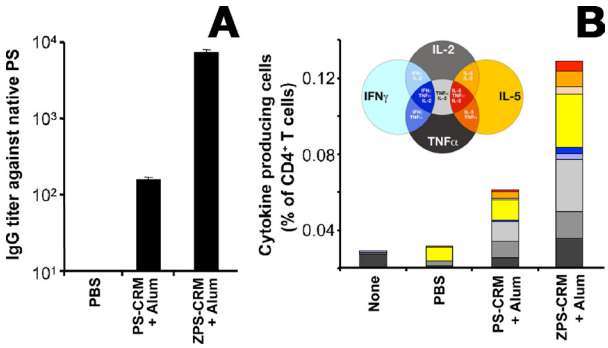

Fig. S7. ZPS-conjugates can be used in combination with alum. (*A*) C57BL/6 mice were immunized i.p. with three doses of the PS-conjugates (1 μg) plus alum (0.4 mg), ZPS-conjugates alone, or ZPS-conjugates (1 μg) plus alum (0.4 mg). PBS was used as negative control. Two weeks post-third dose, sera were analyzed for PS-specific IgG titers. Results are mean of triplicates + SD. (*B*) Spleens from the same mice were tested for $CD4^+$ T-cell cytokine responses to CRM_{197}. Histograms show the total percentage of all cytokine-positive live singlet CD3 + $CD4^+$ cells. Results are obtained from a pool of six mice for each group. This experiment was performed three times with similar results.