Supporting Information

Bessa et al. 10.1073/pnas.1206970109

SI Materials and Methods

Mice and Antigens. To evaluate the Ab response induced by Qβ-virus-like particles (VLP), female, 8-wk-old C57BL/6 mice (Harlan) were immunized either intranasally or subcutaneously with 50 μg of VLP containing *Escherichia coli*-derived ssRNA. TgH(VI10) xYEN mice (1) were bred and maintained at Bio-Support, Zürich. All animal experiments were conducted in accordance with protocols approved by the Swiss Federal Veterinary Office. Qβ-VLPs and AP205 were purified as described elsewhere (2).

Immunization and Pertussis Toxin Treatment. For the intranasal immunization with VLPs, mice were anesthetized with isoflurane and vaccine was administered using a 200-μL pipette. Subcutaneous vaccination was performed by VLP injection into both sides of the abdomen. For both routes of immunization, VLP was diluted in PBS to a final administration volume of 100 μL (2× 50 μL). Mice received pertussis toxin (PTX; 400 μg/ kg) intranvenously 4 h before intranasal VLP administration.

Lung Cell Isolation. For the isolation of lymphocytes from the lung, mice were perfused with 5 mL of PBS in the heart ventricle to clear lungs of blood. Lungs were chopped in small pieces and incubated at 37 °C in media containing collagenase. Finally, lymphocytes were harvested by using a 30% (vol/vol) percoll gradient.

ELISA. For determination of VLP-specific IgG titers, ELISA plates (Nunc Immuno MaxiSorp) were coated overnight with VLPs (1 μg/mL) and ELISA were performed according to standard protocols using HPRO-conjugated goat anti-mouse IgG (Fc γ-specific; Jackson Immunoresearch). Anti-VLP IgG titers in serum are indicated as dilutions reaching half-maximal absorbance at 450 nm.

For determination of free VLP in serum, ELISA plates (Nunc Immuno MaxiSorp) were coated overnight with 100 μL of anti–Qβ-VLP monoclonal Ab (1.5 μg/mL) and quantification of free VLP in serum was determined using biotinylated anti-VLP monoclonal Ab following HPRO-conjugated streptavidin (Jackson Immunoresearch). Optical densities were read in the ELISA reader at 450 nm.

For the detection of allotype-specific antibodies, a mixture of biotin-labeled rat anti-mouse $IgG1^a$ and $IgG2a^a$ or $IgG1^b$ and $IgG2a^b$ (BD Pharmingen) was used followed by streptavidin-HPRO (Jackson Immunoresearch).

ELISPOT Assay. VLP-specific antibody-forming cell (AFC) frequencies were determined as previously described (3). Briefly, 24-well plates were coated with 10 μg/mL VLPs. Splenocytes were added in DMEM containing 2% FCS and incubated for 5 h at 37 °C. Cells were washed off and plates were incubated either with goat anti-mouse IgG (EY Labs), followed by alkaline phosphatase-conjugated donkey anti-goat IgG Ab (Jackson Immunoresearch) before development of alkaline phosphatase color reactions.

Flow Cytometry. Detection of VLP-specific B cells expressing surface Ig was performed by incubation with Alexa 647-conjugated VLP particles. Donor versus host cells were distinguished with a FITC-conjugated anti-CD45.1 (1/300; mouse anti-mouse). Isotype-switched B cells were detected with a mixture of PE-conjugated antibodies at 1/500 dilution [rat anti-IgD, goat anti-IgM serum (Jackson Immunoresearch); hamster anti-CD3, rat anti-CD4, rat anti-CD8, hamster anti-CD11c, rat anti-CD11b, rat anti-Gr-1, and PerCP-Cy5.5-conjugated rat IgG2a anti-CD19 (1/400; 1D3)]. CD62L expression on blood B cells was detected with PE-conjugated rat anti-mouse CD62L. In all cases, Fc-receptors were blocked with rat IgG2b anti-mouse CD16/32 (1/100; 2.4G2). Antibodies were purchased from BD Biosciences, unless otherwise specified.

For detecting cells in association with VLPs in the blood, mice were immunized either intrnasally or subcutaneously with 100 μg of VLP labeled with Alexa-488. Four hours after immunization, mice were killed and blood-cells were stained with antigen-presenting cell-conjugated rat anti-mouse CD19 (BD Pharmingen; 1/400). Fc-receptors were blocked with rat IgG2b anti-mouse CD16/32 (1/100; 2.4G2) and dead cells were excluded by propidium iodide staining.

The B cells expressing VSV-specific BCR were detected by a monoclonal Ab (clone 35.61) recognizing VI10 heavy and light chains (1).

Immunofluorescence. Freshly removed spleens were immersed in OCT and snap-frozen in liquid nitrogen. Tissue sections of 7-μm thickness were cut in a cryostat and fixed with acetone. For detection of VLP-specific B cells, first unlabeled VLPs were added, followed by a polyclonal rabbit anti-VLP antiserum (1/1,500; RCC) and detected with Alexa 488-cojugated goat anti-rabbit Ab (1/1,000). PNA-binding B cells were detected with biotinylated PNA (1/800; Vector Laboratories), followed by Alexa 546-labeled Streptavidin (1/1,000). VLPs were detected by incubating sections with rabbit anti-VLP antiserum (1/1,500; produced by RCC), followed by Alexa 488-conjugated goat anti-rabbit Ab (1/1,000; Molecular Probes). B-cell follicles were identified with Alexa 647 rat anti-mouse B220 (1/200; BD Pharmingen). Lungs were prepared the same way as the spleen, except that they were perfused with 300 μL of OCT prior removal and the sections were 10-μm thick. B cells were detected with unlabeled rat anti-mouse CD19 (1/50; BD Pharmingen), followed by Alexa 546-conjugated goat anti-rat Ab (1/200; Invitrogen). Blood vessels were visualized with Alexa 647-conjugated rat anti-mouse CD31 (1/200; Biolegend). Images were acquired with Axioplan 2 microscope (Zeiss) and pictures were analyzed with Open laboratory software (Improvision).

Immunohistochemistry. Freshly removed spleens were immersed in OCT and snap-frozen in liquid nitrogen. Tissue sections of 7-μm thickness were cut in a cryostat and fixed with acetone. For detection of VLPs, sections were incubated with rabbit anti-VLP antiserum, followed by alkaline phosphatase-labeled goat antibodies to rabbit immunoglobulins (Jackson Immunoresearch) and alkaline phosphatase-labeled donkey antibodies against goat immunoglobulins (Jackson Immunoresearch) (diluted 1/80). Alkaline phosphatase was visualized using Fast Blue substrate Kit III (Vector Laboratories), which yielded a blue precipitate. Red pulp was distinguished from white pulp using Nuclear Fast Red solution (Vector Laboratories).

Radiation Bone-Marrow Chimeras. Radiation bone-marrow chimeras holding vesicular stomatitis virus-specific B cells as well as polyclonal B cells and natural antibodies were generated by intranvenous injection of bone-marrow mixture containing 20% of bone-marrow cells isolated from C57BL/6 Ly5.1 mice and 80% of bone-marrow cells isolated from (VI10)xYEN mice into C57BL/6 Ly5.2 mice that had been lethally irradiated (950 rad γ) 1 d before. Prior to cell transfer, bone-marrow cell suspension was depleted from T cells. After 6 wk, bone-marrow reconstitution was assessed by staining peripheral blood lymphocytes. Thereafter, mice were immunized with 100 μg of VLP intranasally.

Purification and Adoptive Transfer of Lung B Cells. Leukocytes isolated from the lung were incubated with anti-CD19 MACS microbeads (Miltenyi Biotech) and B cells were positively selected

through LS MACS separation columns according to the manufacturer's instructions. Purity of $CD19^+$ B cells was ~90%. Cells were extensively washed with PBS at 4 °C, resuspended in 100 μL of PBS, and injected into the tail vein of a sex-matched recipient. After 10 d, recipient mice were bled for determination of VLP-specific IgG titers.

1. Hangartner L, et al. (2003) Antiviral immune responses in gene-targeted mice expressing the immunoglobulin heavy chain of virus-neutralizing antibodies. *Proc Natl Acad Sci USA* 100(22):12883–12888.
2. Cielens I, et al. (2000) Mutilation of RNA phage Qbeta virus-like particles: From icosahedrons to rods. *FEBS Lett* 482(3):261–264.
3. Gatto D, et al. (2005) Complement receptors regulate differentiation of bone marrow plasma cell precursors expressing transcription factors Blimp-1 and XBP-1. *J Exp Med* 201(6):993–1005.

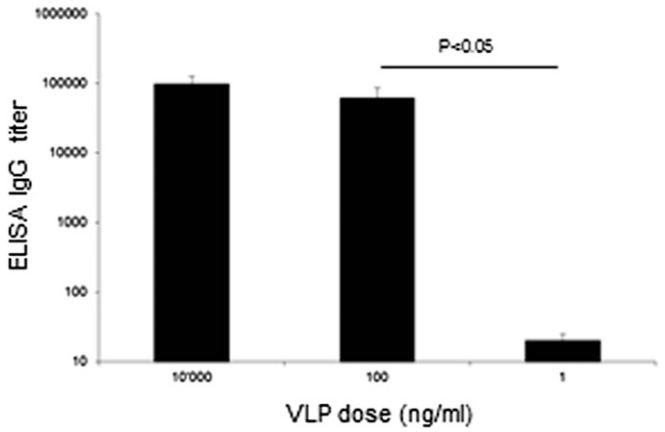

Fig. S1. Low dose of VLPs fails to be immunogenic. C57BL/6 mice were injected intranvenously with the indicated dose of VLPs. VLP-specific IgG titers were determined 10 d later by ELISA (n = 3). P value was calculated by Student t test (two-tailed, unpaired).

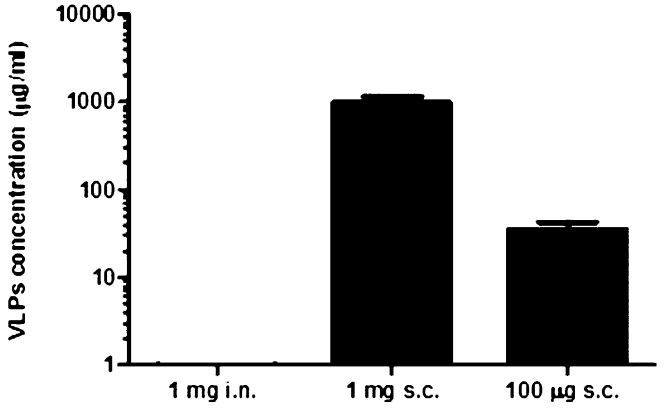

Fig. S2. Quantification of free VLPs in blood after intranasal administration with 1 mg of VLPs. C57BL/6 mice were immunized intranasally with 1 mg of VLPs or subcutaneously with 1 mg or 100 μg of VLPs. Levels of circulating VLPs were determined 7 h later in blood by ELISA.

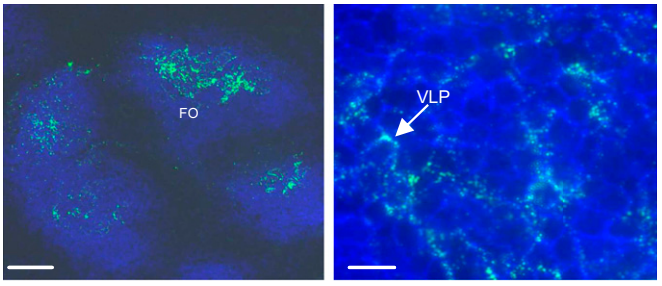

Fig. S3. B cells within the B-cell follicles are associated with VLPs 24 h postintranasal immunization. Histological detection of VLPs on B cells within the splenic follicles 24 h after intranasal administration of 1 mg of VLPs. Blue, B220 staining B cells; green, Alexa 488-conjugated VLPs. Images were made at lower (*Left* scale bar, 100 μm) or higher magnification (*Right* scale bar, 5 μm).

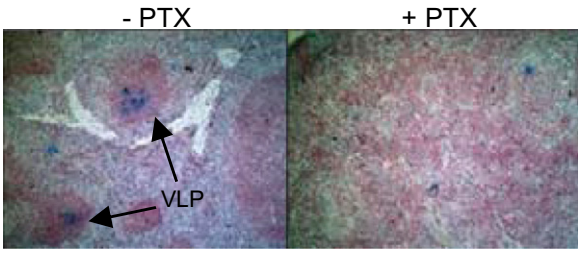

Fig. S4. Mice treated with PTX failed to trap VLPs into splenic follicles. Histological detection of VLPs within splenic B-cell follicles of PTX-treated mice 24 h after intranasal administration of 1 mg of VLPs. Spleen section of untreated mice is shown as control. (Original magnification: 20×.)

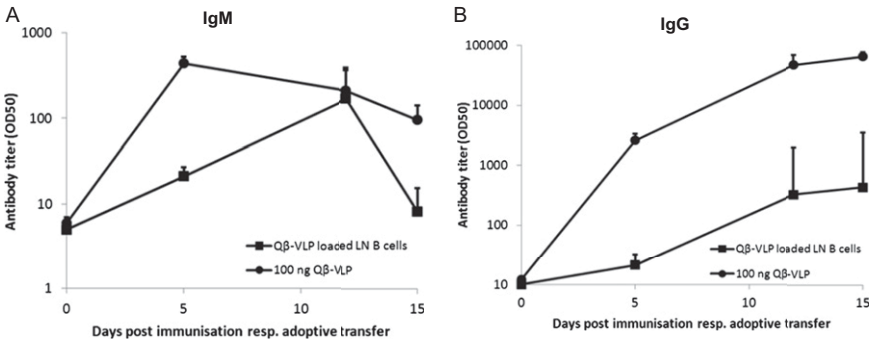

Fig. S5. Induction of humoral response after adoptive transfer of Qβ-VLP loaded B220$^+$ lymph node cells. C57/BL6 mice (n = 3) received 8 × 10^5 MACS-purified B220$^+$ B cells isolated from lymph nodes (0.13% binding Qβ-VLP) that were loaded with 10 μg/mL Qβ-VLP for 30 min in vitro. After the adoptive transfer, mice were bled over time and sera were analyzed for Qβ-VLP specific IgM (*A*) or IgG (*B*) antibodies.

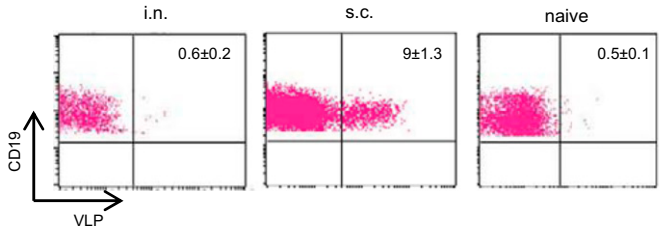

Fig. S6. Intranasal immunization fails to induce B-cell response in nondraining lymph nodes. Mice were immunized intranasally or subcutaneously with 50 μg of VLPs and the frequency of VLP-specific memory B cells in inguinal lymph nodes was assessed 30 d postimmunization. Analysis was performed by gating on isotype-switched B cells (CD19$^+$, IgM, IgD, CD4, CD8, CD11b, and Gr-1$^-$). Mean percentages ± 1 SD of VLP-specific B cells are shown (n = 3).

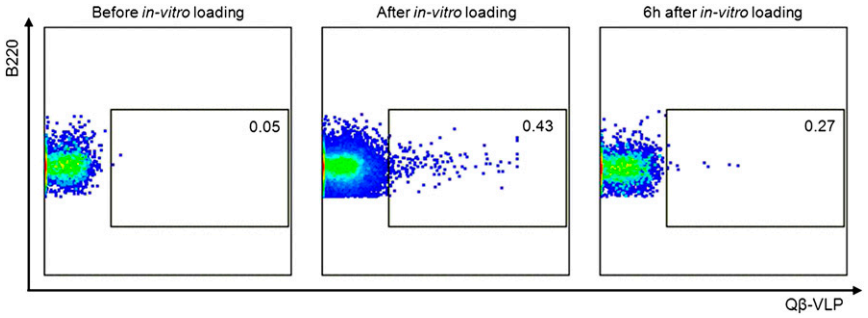

Fig. S7. The release of Qβ-VLPs bound to lung B cells upon in-vitro loading. Lung B220$^+$ B cells were isolated from C57/BL6 mice (n = 3). MACS purified B cells were loaded with 10 μg/mL Qβ-VLPs for 30 min in vitro. Cells were analyzed by flow cytometry for the proportion of Qβ-VLP$^+$ B cells (*Right*) and 6 h after in vitro loading with VLPs.

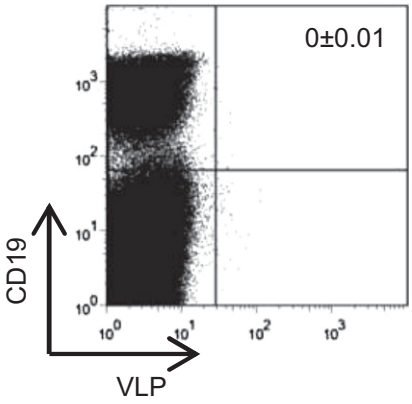

Fig. S8. Sera from WT mice into (VI10)xYEN mice fail to restore the ability of VLP binding into B cells. (VI10)xYEN mice received 200 μL of sera from naïve WT mice intravenously 16 h prior to intranasal immunization with 100 μg of Alexa 488-conjugated VLPs. Analysis of interaction with VLP in blood was determined 4 h later by flow cytometry. Mean percentage ± 1 SD of VLP-binding B cells is indicated (n = 3).

Table S1. Summary of frequency of cells binding intermediate and high amounts of VLPs in blood of WT, FcγRIIB$^{-/-}$, Cr2$^{-/-}$, and (VI10)xYEN mice

VLP	WT	FcγRIIB$^{-/-}$	Cr2$^{-/-}$	(VI10)xYEN
VLP ^{int}	0.1 ± 0.04	0.21 ± 0.07	0.16 ± 0.08	0.02 ± 0.0
VLP ^{high}	0.01 ± 0.0	0.01 ± 0.05	0.08 ± 0.0	0.0 ± 0.0

Analysis was performed 4 h after intranasal administration of 100 μg Alexa 488-conjugated VLPs. Mean values ± SEM (n = 4) are given.