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) intravenously 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 titters in serum are indicated as optical density units (ODU) at 450 nm.

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-labeled goat anti-rabbit IgG (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; BD Pharmingen) were used. 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 VLP-specific B cells, first unlabeled VLPs were added, followed by a polyclonal rabbit anti-VLP antiserum (1/1,500; RCC) and with rabbit anti-VLP antiserum (1/1,500; produced by RCC), followed by Alexa 488-conjugated goat anti-rabbit IgG (1/1,000; Molecular Probes). B-cell follicles were identified with Alexa 488 rat anti-mouse B220 (1/200; BD Pharmingen). Lungs were prepared the same way as the spleen, except that they were perfused with PBS to a final 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 488-conjugated goat anti-rat Ab (1/200; Invitrogen). Blood vessels were visualized with Alexa 488-conjugated rat anti-mouse CD31 (1/200; Biologic). Images were acquired with Axiosoplan 2 microscope (Zeiss) and pictures were analyzed with Open laboratory software (Improvision).

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 intravenous 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 C57BL/6 Ly5.2 mice that had been lethally irradiated (950 rad) 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.

Supporting Information

Bessa et al. 10.1073/pnas.1206970109
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.


**Fig. S1.** Low dose of VLPs fails to be immunogenic. C57BL/6 mice were injected intravenously 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: 20x.)

Fig. S5. Induction of humoral response after adoptive transfer of Qβ-VLP loaded B220+ lymph node cells. C57/BL6 mice (n = 3) received 8 × 10⁵ MACS-purified B220+ 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.
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 naive 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

<table>
<thead>
<tr>
<th>VLP</th>
<th>WT</th>
<th>FcγRIIB−/−</th>
<th>Cr2−/−</th>
<th>(VI10)xYEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLP int</td>
<td>0.1 ± 0.04</td>
<td>0.21 ± 0.07</td>
<td>0.16 ± 0.08</td>
<td>0.02 ± 0.0</td>
</tr>
<tr>
<td>VLP high</td>
<td>0.01 ± 0.0</td>
<td>0.01 ± 0.05</td>
<td>0.08 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
</tbody>
</table>

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