MICE. Mice expressing CFP under the β-actin promoter (Hadjantonakis et al., 2002; 004218; Tg (ACTB-ECFP)) were purchased from The Jackson Laboratory. Cxcr5−/− mice (Forster et al., 1996) were provided by M. Lipp (Max-Delbruck-Center for Molecular Medicine, Berlin, Germany). HEL-specific TCR7 transgenic mice (Neighbors et al., 2006) were provided by A. O’Garra (Medical Research Council, London, UK). Mice deficient for Cr2 (Molina et al., 1996) were provided by J. Atkinson and X. Wu (University of Washington, Seattle, WA).

CELL ISOLATION, CFSE LABELING, AND ADOPTIVE TRANSFER. B and T cells were isolated as previously described (Okada et al., 2005; Phan et al., 2007). For imaging, MD4 B cells were labeled with 2.5 µM CFSE (Invitrogen) as in Phan et al. (2007). For cotransfer of noncognate B cells with MD4 B cells, CD45.1+CD45.2+ B cells from F1 of CD45.1 × CD45.2+ breeders were used to distinguish them from MD4 (CD45.1−CD45.2−) and recipient (CD45.1+CD45.2−) B cells. For analysis of T cell division, 3 × 106 CD45.2+ TCR7 CD4+ T cells were labeled with 4 µM CFSE and cotransferred with 5 × 105 CD45.1+ B cells to CD45.1− recipients. For analysis of GC B cell differentiation, 5 × 105 CD45.1+ B cells and 5 × 105 CD45.2+ TCR7 CD4+ T cells were transferred to CD45.1− recipients.

FLOW CYTOMETRY. For measurement of HEL-PE capture by B cells, single cell suspensions were stained with FITC-conjugated anti-CD19 (1D3; BD) plus Pacific blue–conjugated anti-CD45.2 (clone 104) or anti-CD45.1 (A20; both obtained from BioLegend). For analysis of B cell activation, cell suspensions were also stained with Alexa Fluor 647–conjugated anti-CD86 (GL-1) or anti-CD83 (Michel-19; both obtained from BioLegend). For detecting BP-3 and CD35 on transferred B cells, the BP-3 antibody against CD157 (hybridoma provided by M. Cooper, University of Alabama, Tuscaloosa, AL) and anti-CD35 (8C12; BD) were conjugated with Alexa Fluor 647 using the antibody labeling kit obtained from Invitrogen. For analysis of GC B cell differentiation, cells were stained with PE-Cy7–conjugated anti-IgD (11-26c.2a; BD), PE-Cy5.5–conjugated anti-B220 (RA3-6B2; BD), Pacific blue–conjugated anti-CD45.1, FITC-conjugated anti-IgD (11-26c.2a; BD), and PE-Cy7–conjugated anti-Fas (Jo2; BD). T cell division was assessed by CFSE dilution upon staining cell suspensions with Alexa Fluor 647–conjugated anti-CD45.2 (clone 104; BioLegend) and Alexa Fluor 405–conjugated anti-CD4 (RM4-5; Invitrogen).

IMMUNOFLOURESCENT MICROSCOPY. Tissue sections 5 µm in thickness were stained with Alexa Fluor 647–conjugated anti-B220 (RA3-6B2; BD) or anti-IgD (11-26c.2a; BD) and were detected with AMCA-conjugated anti–rat IgG (Jackson Immunoresearch Laboratories). Sections were then blocked with 5% normal rat serum before staining with Alexa Fluor 647–conjugated anti-CD3 (1G7; BD) or Alexa Fluor 405–conjugated anti-CD4 (RM4-5; Invitrogen) and Alexa Fluor 405–conjugated anti-CD4 (RM4-5; Invitrogen).

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Figure S1. Punctate HEL-PE deposition on FDCs. Magnified image of an LN section prepared as in Fig. 1 A. Bar, 50 µm. Data are representative of follicles from six inguinal LNs in three experiments.
Figure S2. Expression of FcγRIIb and GL-7 in follicles. LN section showing distribution of HEL-PE and staining for IgD (blue), FcγRIIb, or GL-7 (green) at the indicated time points after HEL-PE immunization. Bar, 200 µm. Data are representative of follicles from at least four inguinal LNs at each time point in two experiments.
Figure S3. Cognate T cell proliferation and GC B cell differentiation induced by HEL-PE immunization. (A) Division of TCR7 CD4+ T cells cotransferred with the indicated types of B cells was determined by CFSE dilution 3 d after transfer. Plots were pregated for CD4+CD45.2+ cells. Numbers in the plots indicate percent of dividing cells. (B) Flow cytometric analysis for IgDloFaslo GC B cells 5 d after transfer. Numbers in the plots indicate percent of gated cells. Data are representative of two experiments.
Figure S4. Distribution of B cell turning angles. Turning angles were calculated for CFSE+ MD4 B cells or CFP tg noncognate B cells that were contacting HEL-PE+ FDC processes (in contact, dark shading) or were not contacting such processes (not in contact, light shading). Median turning angles are indicated by numbers and arrowheads. The histogram represents data pooled from three experiments.
Figure S5. Antigen capture and activation of Cr2−/− MD4 B cells. Flow cytometric analysis of wild-type (WT) or Cr2−/− MD4 B cells 12 h after transfer to mice that had been immunized with HEL-PE or DEL-PE. (A–D) Flow cytometry (A and C) and summary of antigen capture and activation marker expression (B and D) are shown. Plots were pregated for CD19+CD45.2+ B cells. Numbers in quadrants in A and C indicate percentage of gated cells. Each point in B and D indicates a single LN. Bars indicate means. Data are from two experiments.
Video 1. Real-time imaging of B cell migration in the FDC network (90 min). Time-lapse image sequence of 20-µm z projection from an inguinal ILN explant showing that CFSE-labeled MD4 B cells (green) and noncognate CFP-tg B cells (cyan) are migrating through the FDC network visualized with HEL-PE (red). Several MD4 B cells carry HEL-PE at their uropod and are tracked with white dots. Capsule-associated collagen fibers appear blue by second harmonic emission. Elapsed time is shown as hours:minutes:seconds. Data are representative of three experiments.

Video 2. Real-time imaging of antigen capture by cognate B cells from FDCs (example 1, 35 min; example 2, 55 min). Two time-lapse image sequences of 20-µm z projection from inguinal LN explants showing acquisition of HEL-PE (red) by CFSE-labeled MD4 B cells (green; circled in the first few frames) from FDC processes. In both examples, MD4 B cells capture large aggregates of HEL-PE (arrowhead). The antigen capture observed in example 1 is depicted in Fig. 2 C. Some noncognate CFP-tg B cells are observed in cyan with weak fluorescence in the green channel. Elapsed time is shown as hours:minutes:seconds. Data are from two different inguinal LNs representative of three experiments.

Video 3. Real-time imaging of prolonged interaction of B cells with FDCs (example 1, 50 min; example 2, 45 min). Two time-lapse image sequences of 20-µm z projection from inguinal LN explants. These are examples of CFSE-labeled MD4 B cells (green; circled in the first few frames) interacting with FDCs for long periods. The minimum observable contact times of these cells are 40 min in example 1, and 22 and 15 min in example 2. Noncognate CFP-tg B cells are observed in cyan some of which are also weakly detected in the green channel. Elapsed time is shown as hours:minutes:seconds. Data are from two different inguinal LNs representative of three experiments.