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JAB1 Is Essential for B Cell Development and Germinal Center Formation and Inversely Regulates Fas Ligand and Bcl6 Expression

Selina Sitte,* Joachim Gläsner,† Julia Jellusova,‡ Florian Weisel,§ Martina Panattoni,¶ Ruggero Pardi,¶ and André Gessner‡

Jun activation domain-binding protein 1 (JAB1) is a highly conserved protein that was initially identified as a coactivator of AP-1 transcription factors (1). Furthermore, JAB1 (synonym CSN5) is a subunit of the COP9 signalosome (2, 3) that plays a central role in regulating proliferation and apoptosis. Because these processes are decisive for B cell development, we investigated JAB1 functions in B cells by establishing a mouse strain with a B cell-specific JAB1 deletion. We show that JAB1 is essential for early B cell development, because the ablation of JAB1 expression blocks B cell development between the pro-B and pre-B cell stages. Furthermore, JAB1 deletion leads to aberrant expression of the apoptosis-triggering protein Fas ligand in pro-B cells. Concomitantly, B cell-specific overexpression of the antiapoptotic protein Bcl2 partially reverses the block in B cell development; rescued JAB1-deficient B cells reach the periphery and produce protective class-switched Abs after Borrelia burgdorferi infection. Interestingly, B cell-rescued mice exhibit no germinal centers but a striking extrafollicular plasma cell accumulation. In addition, JAB1 is essential for Bcl6 expression, a transcriptional repressor required for germinal center formation. These findings identify JAB1 as an important factor in checkpoint control during early B cell development, as well as in fate decisions in mature Ag-primed B cells. The Journal of Immunology, 2012, 188: 2677–2686.
dependent on Abs (21), and the bacteria express lipoproteins with very strong B cell mitogenic activity (22). During the course of infection, B. burgdorferi disseminate in the organism and are preferentially found in the collagenous connective tissue of heart, joints, and other organs (23). The main clinical outcome of experimental Lyme disease is arthritis, and it depends on the genetic background of the mice: susceptible mice, such as C3H/He mice, develop arthritis, whereas C57BL/6 mice are resistant to it (24). Previously, B cell-deficient mice (Ig6 KO) were analyzed in murine Lyme disease; although they were on an arthritis-resistant C57BL/6 background, they developed persistent arthritis (21).

Given that JAB1 regulates proliferation and apoptosis, crucial processes in B cell development, we addressed the roles of JAB1 in early B cell development by generating B cell-specific JAB1-deficient mice and analyzing B cell development, as well as the humoral immune response, after B. burgdorferi infection. We show that JAB1 is essential for early B cell development and that overexpression of Bcl2 partially rescues this defect. Furthermore, JAB1 is essential for GC formation; thus, we identify JAB1 as an important factor in fate decisions of Ag-primed B cells.

Materials and Methods

Mouse strains
Mice with floxed alleles of JAB1 on a C57BL6 background were described before (9). Mb1-cre mice on a BALB/c background were kindly provided by Michael Reth, Max-Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany (25). Both strains were crossed to selectively delete JAB1 in B cells (JAB1<sup>-del/del</sup>). The Eμ bcl2-22 strain (26) was used to introduce bcl2 as a B cell-specific transgene in rescue experiments. Experiments were performed according to a B cell survival guidelines for animal welfare and legal requirements.

Flow cytometry and Abs
FACS analyses were performed using the following Abs and reagents: B220-PerCP, CD5-biotin, CD19-biotin, CD23-FITC, CD43-PE, CD127 (IL-7R)-PE, CD176 (Gal)-biotin, IgM-biotin (BD), CD19-FITC, CD25-alkaline phosphatase, streptavidin-alkaline phosphatase (Invitrogen), CD21-PE (eBioscience), IgM-FITC (Southern Biotech), and peanut agglutinin (PNA)-FITC (Vector Laboratories), or rabbit anti-Bcl6 (Santa Cruz); washed; and stained for 30 min, with anti–IgM-Cy3 or -Cy5 (Dianova), anti–IgD-biotin (eBioscience), anti-CD178 (FasL)-biotin, IgM-biotin (BD), CD19-FITC, CD25-allophycocyanin, CD5-biotin, CD19-biotin, CD23-FITC, CD43-PE, CD127 (IL-7R)–PE, Cops5; and fr. B/C (B220+, CD43+, BP-1+). CD5-biotin, CD19-biotin, CD23-FITC, and CD43-PE were starved for 1.5 h in Opti-MEM with 0.5% FCS and then pulsed with IL-7 culture and proliferation assay

B cells from bone marrow were isolated by magnetic cell sorting with CD19-coated magnetic beads (Miltenyi Biotec) on an Auto MACS device (Miltenyi Biotec). Cells were cultured in Opti-MEM (Invitrogen) supplemented with the indicated concentrations of 10^-7-containing supernatant from J558L cells (a gift from Dr. F. Melchers, University of Basel). Proliferation was determined by incorporation of ^3H-labeled thymidine after 3 d. For investigation of STAT5 phosphorylation, CD19-enriched B cells were starved for 1.5 h in Opti-MEM with 0.5% FCS and then pulsed with Opti-MEM supplemented with 25 ng/ml rIL-7 and 10% FCS for 15 min. Cells were immediately fixed after the IL-7 pulse.

Cell cycle analysis

Bone marrow cells were stained with Abs, as described in the flow cytometry section. Cells were then fixed with 2% paraformaldehyde at 4 °C. To stain the DNA, cells were incubated with 1 mg/ml DAPI (Sigma) in PBS with 0.01% Tween 20 for 30 min at room temperature. Cells were analyzed on a FACSAria flow cytometer (BD). Duplicates were excluded prior to analysis of DNA content.

ELISA

Ab titers of naive mice were measured by standard ELISA method. Briefly, plates were coated with anti-Ig, and sera were serially diluted 1:3 in PBS/1% BSA. Alkaline phosphatase-conjugated anti-Ig (Southern Biotech) was used for detection.

Serum transfer

Sera from B. burgdorferi-infected WT, JAB1<sup>-del/del</sup>, and Bcl2 JAB1<sup>-del/del</sup> mice were diluted 3-fold in sterile PBS and injected i.p. in a volume of 200 μl into Rag1 KO mice. After 24 h, mice were infected with B. burgdorferi. At day 28 postinfection (p.i.), pathogen burden was determined, as described previously (27).

Results

JAB1<sup>-del/del</sup> mice exhibit a block in early B cell development

To examine the role of JAB1 in B cell development, we generated B cell-specific JAB1 KO mice. A mouse strain expressing the cre recombinase under the control of the mbl promoter on a BALB/c background (25) was crossed with a mouse strain with floxed alleles of JAB1 on a C57BL/6 background (9). In the experiments, we compared littermates heterozygous for mbl-cre (designated as JAB1<sup>−del/del</sup>) or lacking mbl-cre (designated as wild-type [WT]). First, we performed FACS analyses of peripheral organs of WT and JAB1<sup>−del/del</sup> mice to investigate whether the B cell compartment is...
affected by JAB1 deletion. Peripheral B cells were absent in JAB1\textsuperscript{del/del} mice: no immature B cells were found in the spleen (Fig. 1A), both B1a (CD5\textsuperscript{+}) and B1b (CD5\textsuperscript{−}) B cells were missing from the peritoneum, and no B cells were detected in blood (data not shown). T cell subsets were not altered (data not shown).

Next, we analyzed bone marrow of WT and JAB1\textsuperscript{del/del} mice to identify the stage at which the B cell development is blocked. We observed that the pro-B cell compartment (B220\textsuperscript{high}/CD43\textsuperscript{+}) and the portion of very early pro-B cells (B220\textsuperscript{+/}ckit\textsuperscript{+}) were relatively enlarged in JAB1\textsuperscript{del/del} mice (Fig. 1B, upper panels). However, the pre-B cell portion (CD19\textsuperscript{+/}CD25\textsuperscript{+}) was reduced in JAB1\textsuperscript{del/del} mice. Moreover, a small portion of the remaining B cells in JAB1\textsuperscript{del/del} mice were intracellularly positive for IgM H chain, indicating that few B cells reached the pre-B cell stage (Fig. 1B, lower panels). We conclude that, in JAB1\textsuperscript{del/del} mice, B cell development is blocked between the late pro-B cell and pre-B cell stages.

Histology of spleen sections confirmed the total absence of peripheral B cells in JAB1\textsuperscript{del/del} mice (Fig. 1C). WT spleens displayed normal architecture, with regularly formed follicles surrounded by the marginal zone (MZ) and IgM-high\textsuperscript{+} plasma cells dispersed in the red pulp. In contrast, spleens from JAB1\textsuperscript{del/del} mice showed a strongly disturbed architecture without either B cell-containing follicles or dispersed plasma cells.

**FIGURE 1.** JAB1\textsuperscript{del/del} mice have a block in early B cell development. (A and B) Flow cytometry was used to compare B cell numbers in WT and JAB1\textsuperscript{del/del} mice. Lymphocytes were pregated based on their forward/ side scatter profile. Numbers refer to percentage values of the respective B cell subset out of total lymphocytes. Blots are representative of at least four independent experiments each, with at least three mice per group. (A) Spleen cells (left panels) and peritoneal exudate cells (PEC; right panels) were stained with B cell-specific Abs. (B) Bone marrow cells were stained with Abs that identify different developmental B cell stages. μ H chain was stained intracellularly. Pro-B cells: B220\textsuperscript{+/}CD43\textsuperscript{+}; early pro-B cells: B220\textsuperscript{+/}ckit\textsuperscript{+}; pre-B cells: CD19\textsuperscript{+/}μ H chain\textsuperscript{−} and CD19\textsuperscript{+/}CD25\textsuperscript{−}. (C) Splenic cryosections were stained with anti-sialoadhesin (MOMA-1; red) to identify metallophilic macrophages and anti-IgM (green) to identify B cells. Scale bar, 500 μm.

**Aberrant FasL expression in JAB1-deficient pro-B cells**

We then analyzed the gene expression of JAB1-deficient pro-B cells. Although mbl-cre mediates deletion very early in B cell development (25), we proposed that efficient JAB1 deletion might not occur until the late pro-B cell stage. Therefore, pro-B cells of WT and JAB1\textsuperscript{del/del} mice were separated by cell sorting into fr. A and fr. B/C (28). Cells of fr. A represent early pro-B cells, and cells of fr. B/C represent late pro-B cells. In JAB1\textsuperscript{del/del} pro-B cells of fr. B/C, the JAB1 mRNA level was reduced to 30% compared with the WT level; however, in pro-B cells of fr. A, the JAB1 mRNA level was comparable between WT and JAB1\textsuperscript{del/del} cells (Fig. 2A). Previously, p53 was shown to accumulate in JAB1-deficient blastocytes (8). Therefore, expression of p53 target genes in pro-B cells of fr. B/C was analyzed. We found that Fasl mRNA, a p53 target gene (29), was not detectable in WT cells but was highly expressed in JAB1\textsuperscript{del/del} cells (data not shown). This finding was confirmed on the protein level by flow cytometry: WT pro-B cells of fr. B/C expressed no surface FasL, whereas the majority of JAB1\textsuperscript{del/del} cells showed surface expression of FasL (Fig. 2B). However, expression of additional known target genes of p53 were not or only marginally changed, as determined by real-time RT-PCR (Supplemental Fig. 1).
**FIGURE 2.** JAB1-deficient B cells show aberrant FasL expression. (A) JAB1 mRNA levels in sorted pro-B cells of WT and JAB1del/del mice measured by quantitative RT-PCR. Cells of fr. A represent early pro-B cells, and cells of fr. B/C represent late pro-B cell. (B) FasL surface expression of fr. B/C pro-B cells of WT and JAB1del/del mice determined by flow cytometry. Pro-B cells fr. B/C (B220+, CD43+, CD24+) were pregated. Graphs are representative of at least five mice analyzed per group.

JAB1del/del mice develop persistent arthritis after B. burgdorferi infection

We also analyzed JAB1del/del mice in the murine model of Lyme disease, because we hypothesized that putative residual B cells in JAB1del/del mice would be activated and massively expanded by *B. burgdorferi*, an extremely strong B cell mitogen (22). Therefore, to exclude the presence of functional relevant numbers of residual B cells in JAB1del/del mice, WT and JAB1del/del mice were infected with *B. burgdorferi* to induce Lyme disease. The clinical outcome of disease was monitored by measurement of joint swelling that normally emerges first at the site of inoculation and eventually affects the contralateral site if inflammation persists. WT and JAB1del/del mice were on an arthritis-resistant genetic background. By day 7 p.i., JAB1del/del mice displayed arthritis of the tibiotarsal joints of the inoculated (right) site, whereas the contralateral joints were still unaffected (Fig. 3A). By day 22 p.i., the left joints of JAB1del/del mice also developed arthritis, which persisted for \( \geq 114 \) d. In contrast, in WT mice, we observed only a moderate swelling of the right joints; the left joints were not affected. The pathogen burden was comparable in WT and JAB1del/del mice at day 22 p.i. (Fig. 3B). At later time points (days 129 and 162 p.i.), JAB1del/del mice showed a strongly increased pathogen burden, whereas in WT mice, *Borrelia* numbers were just above the detection limit. The pathogen burden was also very high in the hearts of JAB1del/del mice and was not detectable in WT hearts (data not shown). Because the clearance of *B. burgdorferi* is dependent on Abs (21), we measured *B. burgdorferi*-specific Ab titers in sera of infected mice. In WT sera, *B. burgdorferi*-specific Abs of the classes IgM, IgG1, and IgG2a were detected, whereas in sera of JAB1del/del mice, no *B. burgdorferi*-specific Abs were found (Fig. 3C). Nevertheless, JAB1del/del mice mounted a strong antibacterial Th1 cell response, similar to that of WT mice (data not shown). Taken together, these findings clearly demonstrate that JAB1del/del mice suffered from persistent Lyme disease, emphasizing the complete B cell defect in these mice.

**B cell development is partially rescued by B cell-specific Bcl2 overexpression**

We next asked whether the observed block in B cell development of JAB1del/del mice might be rescued by antiapoptotic strategies. Bcl2 is an antiapoptotic protein that inhibits the intrinsic apoptosis pathway (30, 31). Therefore, we crossbred the JAB1del/del mouse strain with a mouse strain overexpressing human Bcl2 as a B cell-specific transgene (26) and designated the new mouse strain Bcl2 JAB1del/del. First, bone marrow of WT, JAB1del/del, and Bcl2 JAB1del/del mice were analyzed by flow cytometry to follow early B cell development. The overall percentage of CD19+ cells did not differ between WT and Bcl2 JAB1del/del mice (Fig. 4A, upper panels). However, in Bcl2 JAB1del/del bone marrow, the frequency of pre-B cells (\( \mu \) H chain) was reduced \( \sim 50\% \) compared with WT bone marrow, but it was 10-fold higher than in JAB1del/del bone marrow; the remaining CD19+ cells represented pro-B cells (\( \mu \) H chain). In Bcl2 JAB1del/del mice, the frequencies of early and immature B cells were comparable to WT mice, whereas the frequency of T1 B cells was reduced 6-fold compared with WT mice, and recirculating B cells were almost completely missing (Fig. 4A, lower panels).

These findings prompted us to analyze the peripheral B cell compartments of Bcl2 JAB1del/del mice to investigate the distri-
bution of rescued B cells in the periphery. In blood and spleen of Bcl2 JAB1del/del mice, we indeed found immature IgM+ B cells; however, their frequencies were reduced 3- and 16-fold, respectively, compared with WT frequencies (Fig. 4B, upper panels). In addition, IgM^2 B cells were found in blood and spleen of Bcl2 JAB1del/del mice. Those cells were also detected in the Bcl2-overexpressing mouse strain without JAB1 deletion (data not shown) and might represent immature progenitor B cells escaping from the bone marrow. In contrast, the numbers of peritoneal B cells and MZ B cells in the spleen were not corrected by Bcl2 overexpression (Fig. 4B, lower panels). The MZ B cell defect of our Bcl2 JAB1del/del strain is in line with the results of a previous
Rescued peripheral B cells lack JAB1 protein but show aberrant FasL expression

Next, we asked whether the rescued B cells indeed lack the JAB1 protein to exclude the possibility that Bcl2 JAB1del/del cells, which might have escaped mb1-cre-mediated deletion, could have accumulated in the periphery because of prevented apoptosis. An immunoblot analysis of sorted splenic B cells from WT and Bcl2 JAB1del/del splenocytes demonstrated that JAB1 protein was completely absent in Bcl2 JAB1del/del B cells, whereas human Bcl2 was highly expressed in these rescued B cells (Fig. 5A). Therefore, we confirmed successful JAB1 deletion in rescued B cells at the protein level. Interestingly, murine Bcl2 was not detectable in the rescued B cells (data not shown), which might be the result of an autorregulatory feedback suppression by overexpressed human Bcl2.

We next asked whether the aberrant FasL expression of JAB1del/del pro-B cells (Fig. 2B) was compensated for in rescued peripheral B cells. Surprisingly, splenic B cells of Bcl2 JAB1del/del mice showed enhanced FasL surface expression compared with WT B cells (Fig. 5B). Thus, Bcl2 overexpression does not abrogate aberrant FasL expression caused by JAB1 deletion.

To test the proliferative capacity of the rescued B cells in vitro, bone marrow B cells were cultured in the presence of IL-7. WT bone marrow B cells proliferated upon IL-7 stimulus in a dose-dependent manner, whereas JAB1del/del and Bcl2 JAB1del/del cells showed no proliferation (Fig. 5C), and IL-7R was expressed at similar levels on B cells of each genotype (Supplemental Fig. 3). STAT5 phosphorylation was analyzed to investigate the underlying mechanism of the observed proliferation defect. STAT5 is the key transcription factor mediating IL-7 signaling in developing B cells (34). STAT5 phosphorylation in bone marrow B cells was analyzed by flow cytometry with a phosphorylation-specific STAT5 Ab after a short IL-7 pulse. Stimulation of WT cells leads to a small, but detectable, signal of STAT5 phosphorylation compared with unstimulated cells (Fig. 5D). JAB1del/del and Bcl2 JAB1del/del cells also exhibited STAT5 phosphorylation after IL-7 stimulation. This indicates that the deletion of JAB1 affects IL-7 signaling downstream of STAT5.

JAB1 deletion leads to G2/M phase cell cycle arrest

Because JAB1 was shown to have a huge impact on the cell cycle, we performed cell cycle analyses of bone marrow B cells. In the case of pre-B cells (intracellularly positive for IgM H chain = μH), the cell cycle phases were distributed similarly in WT and Bcl2 JAB1del/del cells (85.1% and 82.1% for G0/G1 phase; 7.3% and 6.5% for S phase; 6.9% and 10.9% for G2/M phase) (Fig. 6A). In contrast, fewer JAB1del/del cells were in G0/G1 phase (53.4%) and more were in S phase (23.5%) and G2/M phase (22.7%) compared with WT cells. This indicates that JAB1del/del cells are arrested in S/G2/M phase. In the case of immature B cells (surface IgM+), 95.3% of WT cells were in G0/G1 phase, and 1.7% were in G2/M phase (Fig. 6B). Interestingly, only 84.9% of Bcl2 JAB1del/del cells were in G0/G1 phase, and 9.6% were in G2/M phase. The percentage of cells in S phase was not different between WT and Bcl2 JAB1del/del cells (1.9% and 3.2%, respectively). Thus, rescued B cells seem to be arrested in G2/M phase.

Ab production is independent of JAB1

To investigate whether the rescued B cells are functional in terms of Ab production, we measured serum Ig concentrations of naive WT, JAB1del/del, and Bcl2 JAB1del/del mice by ELISA. On average, in WT sera, the concentrations were 791 μg/ml for IgG2b (Fig. 7). In sera from JAB1del/del mice, the concentrations were 298 μg/ml for IgM, 800 μg/ml for IgG1, and 791 μg/ml for IgG2b. In sera from Bcl2 JAB1del/del mice, we performed immunofluorescence staining of spleen sections. WT spleens showed a normal architecture, whereas JAB1del/del spleens contained no B cells (Supplemental Fig. 2). Interestingly, in Bcl2 JAB1del/del spleens, we detected neither follicular nor MZ B cells, but IgM+ cells were dispersed in the red pulp. Those cells probably represented plasma cells. The absence of MZ B cells in Bcl2 JAB1del/del spleens correlates with the results of our FACS analyses in which we detected no MZ B cells (Fig. 4B, lower panels). In addition, fewer metallophilic macrophages (MOMA-1+) were present in spleens of both JAB1del/del and Bcl2 JAB1del/del mice. The reduced number of metallophilic macrophages in spleens of JAB1del/del and Bcl2 JAB1del/del mice is in line with the results of previous studies showing that B cells are crucial for the development of MZ cells, including metallophilic macrophages (33).
mice, very low concentrations of Igs were detected (3 μg/ml IgM, 19 μg/ml IgG1, and 2 μg/ml IgG2b). In contrast, sera from Bcl2 JAB1del/del mice showed elevated Ig concentrations (16 μg/ml IgM, 148 μg/ml IgG1, and 35 μg/ml IgG2b). The very low Ig concentrations in sera of some JAB1del/del mice might be produced either by B cells that escaped the cre-mediated JAB1 deletion and/or, in the case of IgG1 and IgG2b, represent residual maternal Abs. These data indicate that Ab production of naive B cells is independent of JAB1.

Bcl2 JAB1del/del mice produce B. burgdorferi-specific protective Abs

To further investigate the immunological capacities of the rescued JAB1-deficient B cells, we infected Bcl2 JAB1del/del mice with B. burgdorferi. The clinical outcome of Lyme arthritis did not differ between JAB1del/del and Bcl2 JAB1del/del mice; both strains developed persistent arthritis affecting the right and left tibiotarsal joints (data not shown). However, Bcl2 JAB1del/del mice produced B. burgdorferi-specific Abs at day 29 p.i., albeit at reduced levels compared with WT mice, whereas JAB1del/del mice did not produce B. burgdorferi-specific Abs (Fig. 8A). The B. burgdorferi-specific Abs of Bcl2 JAB1del/del mice were not able to efficiently control the pathogen burden in the joint or heart tissue of Bcl2 JAB1del/del mice (Fig. 8B), which might result from the relatively low titers during the early course of infection (e.g., day 4) in these animals (Fig. 8A). To examine whether the B. burgdorferi-specific Abs of Bcl2 JAB1del/del mice have antibacterial protective capacity if already present at the onset of infection, sera of B. burgdorferi-infected Bcl2 JAB1del/del mice were transferred into Rag1 KO mice prior to infection. The pathogen burden was determined 4 wk p.i. Rag1 KO mice that had received no serum or serum from JAB1del/del mice showed a high bacterial burden in the inoculated right tibiotarsal joints (Fig. 8C). In contrast, no B. burgdorferi DNA was detected in the joints of Rag1 KO mice, which had received serum from WT or Bcl2 JAB1del/del mice. As demonstrated by these results, Borrelia-specific Abs produced by JAB1-deficient B cells are protective against B. burgdorferi after transfer into immunodeficient mice. Therefore, the lack of endogenous protection in Bcl2 JAB1del/del mice is most likely a consequence of too few B cells and subthreshold levels of antibacterial Abs during the early dissemination phase of the pathogen.

JAB1 is essential for GC formation, as well as the induction of Bcl6 and AID expression

To further characterize the Bcl2-overexpressing, JAB1-deficient B cells, we studied GC formation after B. burgdorferi infection. First, immunofluorescence analyses of spleen sections of infected mice were performed. In WT spleens, GCs (PNA+, IgD+) were detected at day 84 p.i. (Fig. 9A). In contrast, the spleens of JAB1del/del mice lacked all B cells, consistent with the above-mentioned B cell defect of these mice. Strikingly, spleens of Bcl2 JAB1del/del mice contained no GCs but showed massive extrafollicular accumulation of IgM+ cells. We performed flow cytometry analysis to confirm this finding. In WT spleens, 0.5% GC B cells were identified by staining with PNA, whereas JAB1del/del mice contained no GCs but showed massive extrafollicular accumulation of IgM+ cells. We performed flow cytometry analysis to confirm this finding. In WT spleens, 0.5% GC B cells were identified by staining with PNA, whereas, in contrast, no GC B cells were detected in Bcl2 JAB1del/del spleens (Supplemental Fig. 4). Therefore, we conclude that in the absence of JAB1, specific antibacterial humoral immunity is generated solely extrafollicularly. The defect in GC formation is not caused by Bcl2 overexpression, because the original Bcl2-transgenic mouse strain shows normal GC formation after immunization (35).
Because the transcriptional repressor Bcl6 plays an essential role in GC formation (15–17), we investigated Bcl6 expression in sorted splenic B cells of WT and Bcl2 JAB1del/del mice after immunization with SRBCs. The Bcl6 transcript level at day 10 after immunization was identical in WT and Bcl2 JAB1del/del mice (Fig. 9B). In contrast, Bcl6 protein was absent in Bcl2 JAB1del/del cells, but it was clearly detectable in WT cells (Fig. 9C). Another GC-specific transcript, Aidca (encoding AID) (36), was downregulated 10-fold in B cells of Bcl2 JAB1 mice after SRBC immunization and upregulated 37-fold in WT GC B cells compared with WT non-GC B cells (Fig. 9B).

To further examine Bcl6 protein expression, we performed immunofluorescence staining of spleen sections after B. burgdorferi infection. Previous studies showed that Bcl6 protein expression is restricted to B cells and CD4+ follicular Th cells within the GCs (37). In WT spleens, we found a dotted Bcl6 staining restricted to the GCs (Fig. 9D). In contrast, in spleens of Bcl2 JAB1del/del mice, very few cells within the follicle were positive for Bcl6 staining, probably representing follicular Th cells (Fig. 9D, arrows). We conclude that the absence of Bcl6 protein and Aidca mRNA in JAB1-deficient Bcl2-overexpressing B cells lead to the observed defect in GC formation.

### Discussion

We investigated JAB1 functions in B cells by cell type-specific deletion and found that JAB1 is essential for early B cell development. The B cell development is blocked between late pro-B and pre-B cell stages, and no peripheral B cells were found (Fig. 1).
This is in line with previous results showing that JAB1 is important for thymocyte development (9). T cell-specific JAB1 deletion resulted in partial impairment of thymocyte development and a notable fraction of T cells emerges at the periphery. In contrast, in our B cell-specific JAB1 KO mice, peripheral B cells were absolutely absent, presumably due to the high efficiency of mb1-cre–mediated deletion (25).

Gene expression analysis of JAB1-deficient pro-B cells revealed that JAB1 suppresses FasL expression (Fig. 2). It was shown that JAB1 deficiency causes accumulation of p53, although proliferative defects observed in JAB1-deficient cells were not dependent on functional p53 (38, 39). In JAB-deficient B cell precursors we detected no or only marginally changed expression levels of several p53-dependent target genes, whereas Fasl was markedly upregulated. So we propose that Fasl be defined as a novel p53-independent JAB1 target protein. Fasl is involved in the major apoptosis pathway in immune cells triggering cell death of Fas-bearing cells (11). Furthermore, reverse signaling by Fasl was shown to activate multiple signaling molecules in T cells, which leads to apoptosis or survival, depending on cell type and conditions (40). It is tempting to speculate that reverse FasL signaling contributes to the termination of B cell development in the absence of JAB1. Interestingly, Kırkin et al. (41) observed an 11-kDa Fasl intracellular domain (ICD), which was generated via sequential proteolysis and subsequently translocates to the nucleus where it modulates gene transcription. Furthermore, the analysis of knockin mice lacking the Fasl ICD revealed that plasma cell numbers, generation of GC B cells, and, consequently, production of Ag-specific Abs in response to immunization with T cell-dependent or T cell-independent Ag are negatively regulated by Fasl ICD-dependent signals (42). Because of the lack of suitable reagents allowing the complete functional blockade of Fasl, including retrograde signaling by Fasl ICD in vivo, we have initiated mouse breedings to generate Fasl-deficient mice with a B cell-specific JAB1 deletion to address the role of overexpressed Fasl during B cell development.

Overexpression of the antiapoptotic protein Bcl2 led to partial rescue of the block in B cell development. B cells, which lack JAB1, reach the periphery. To our knowledge, this shows for the first time that cells can develop and survive without JAB1.

Interestingly, the aberrant FasL expression of JAB1-deficient pro-B cells is not abrogated by Bcl2 overexpression, because Fasl is highly expressed on the surface of rescued peripheral B cells (Fig. 5B). It remains to be investigated whether this enhanced Fasl expression might lead to enhanced apoptosis of Fas+ non-B cells in the steady state or during an immune response in the B cell-rescued mice.

Neither isolated rescued pro-B cells nor the JAB1del/del cells proliferate in vitro after IL-7 stimulus, demonstrating a nonredundant role for JAB1 in IL-7–driven proliferation in vitro. As analysis of STAT5 phosphorylation revealed, the signaling defect in the absence of JAB1 is downstream of STAT5. However, in vivo, where factors in addition to IL-7 influence the developing B cells (43), some of the signals might operate in the absence of JAB1 when Bcl2 is overexpressed. In fact, analysis of the cell cycle revealed that JAB1-deficient pre-B cells are arrested in S/G2/M phase, whereas rescued pre-B cells proceed normally in the cell cycle (Fig. 6A). At the subsequent developmental stage, immature rescued B cells also show abnormal cell cycle phase distribution; rescued B cells are arrested in G1/M phase. We hypothesize that, in rescued B cells, the defects caused by JAB1 deletion become evident later than in JAB1del/del cells. The findings of the cell cycle analyses correlate with those in JAB1-deficient T cells, which are arrested in S/G2/M phase (9). Thus, JAB1 is an important cell cycle regulator in developing lymphocytes.

JAB1 is not essential for the proper immunological function of B cells, as the Ab titers in sera of rescued mice implicate (Fig. 7). In addition, these rescued B cells can produce Ag-specific protective Abs, indicating a dispensable role for JAB1 in specific humoral immune responses. These findings extend those previously reported for T cells (9), because in that study, the functional properties of the rescued T cells were not analyzed.

Strikingly, the Abs produced by rescued B cells originate solely from extrafollicular plasma cells; no GCs were found in these mice (Fig. 9). The mechanisms determining extrafollicular versus GC immune responses are largely unclear (20). Our results suggest that JAB1 might be a crucial factor in this decision. No Bcl6 protein was detected after immunization in rescued B cells (Fig. 9C, 9D), whereas the transcript level was similar to WT. To our knowledge, our findings demonstrate for the first time that JAB1 mediates the previously described posttranscriptional regulation of Bcl6 (19). It remains to be investigated whether JAB1 enhances Bcl6 translation and/or protein stability. A recent study showed a critical role for Bcl6 in early B cell development (44). We speculate that the observed B cell development block might be partially caused by a putative absence of Bcl6 in JAB1-deficient early B cells.

In summary, we demonstrate that JAB1 is essential for early B cell development and that Bcl2 overexpression can partially rescue the JAB1-deletion phenotype. Secretion of antibacterial class-switched Igs is not JAB1 dependent, whereas JAB1 is indispensable for GC formation. Furthermore, we identified Fasl and Bcl6 as novel target proteins of JAB1-dependent regulation.

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Disclosures
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References