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The B-Cell-Specific src-Family Kinase Blk Is Dispensable for B-Cell Development and Activation

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The B-cell lymphocyte kinase (Blk) is a src-family protein tyrosine kinase specifically expressed in B-lineage cells of mice. The early onset of Blk expression during B-cell development in the bone marrow and the high expression levels of Blk in mature B cells suggest a possible important role of Blk in B-cell physiology. To study the in vivo function of Blk, mice homozygous for the targeted disruption of the *blk* gene were generated. In homozygous mutant mice, neither *blk* mRNA nor Blk protein is expressed. Despite the absence of Blk, the development, in vitro activation, and humoral immune responses of B cells to T-cell-dependent and -independent antigens are unaltered. These data are consistent with functional redundancy of Blk in B-cell development and immune responses.

Activation of B cells by various ligands is accompanied by activation of src-family protein tyrosine kinases (PTKs). Cross-linking of the B-cell receptor (BCR) leads to the activation of src-family PTKs Blk, Fyn, and Lyn (22). In addition, Lyn can be activated by antibody-mediated cross-linking of CD19 and, to a lesser extent, of RP-105 (3), whereas Fyn is part of the interleukin-5 receptor signal-transducing complex (2, 26). Activation of src-family PTKs precedes and is probably required for the activation of PTK Syk (13, 21), which belongs to the ZAP-70/Syk family of PTKs and is essential for pre-BCR and BCR-mediated B-cell development in the bone marrow (5). The src-family PTKs also trigger the phosphorylation and activation of the Tec-homologous kinase Btk, which plays a critical role in B-cell survival (1) and antigen-induced B-cell activation (7, 23).

The role of src-family PTKs in B-cell function in vivo remains largely elusive. A deficiency in Lyn decreases the threshold for BCR-mediated B-cell activation but renders B cells unresponsive to antibody-mediated cross-linking of RP-105 (3). Abnormal signalling properties of Lyn-deficient B-lineage cells do not significantly affect B-cell development in the bone marrow but are probably responsible for an autoimmune disease associated with high titers of anti-DNA and anti-nuclear antibodies in the blood of the mutant mice (4, 9, 18). The deficiency in Fyn has no significant effect on B-cell development and activation, with the exception of causing diminished B-cell responses to interleukin-5 (2, 26). In contrast to Lyn and Fyn, which are expressed in cells of different hematopoietic lineages, Blk is the only src-family PTK specifically expressed in B-lineage cells of mice (6). The expression of Blk starts at the late pro-B-cell, early pre-B-cell stage of B-cell development and remains constantly high at later stages of B-cell maturation (24). These data, as well as the induction of malignant transformation of B-cell progenitors by the expression of constitutively active Blk (16), suggest a possible involvement of Blk in the control of B-lineage cell differentiation and proliferation. On the other hand, suppression of the surface immunoglobulin M (IgM)-mediated apoptosis of B-lymphoma cells by Blk antisense oligonucleotides points to a role for Blk in negative selection of B cells (25). To define the role of Blk in B-cell development and activation, we have analyzed B-cell development and function in Blk-deficient mice.

MATERIALS AND METHODS

Construction of the *blk* targeting vector. The fragment of the *blk* gene containing a part of exon 8 (which encodes amino acids (aa) 285 to 311), intron 8, and a part of exon 9 (encoding aa 312 to 333) was amplified by PCR from C57BL/6 genomic DNA and used as a short arm of homology. Primers 5′ CTG CAG CAT GAG AGG CTG GTT CG 3′ (aa 285 to 292; direct PCR primer) and 5′ GTC AAT CAG CCT TGG AAG GGA C 3′ (aa 327 to 333; reverse PCR primer) were used for exons 8 and 9, respectively. The short arm of homology was cloned into the *Xho*I site of plasmid pTV-0 (B. Walter and A. Tarakhovsky, unpublished data). This plasmid carries both the neomycin resistance (Neo^r) and the herpes simplex virus thymidine kinase (HSV TK) genes. The polylinker containing *Cla*I, *Not*I, *Xba*I, and *Xho*I is located 5′ of the Neo^r gene, while a polylinker containing *Bam*HI, *Hpa*I, *Nhe*I, and *Sal*I is positioned 3′ of the Neo^r gene. The 8.3-kb *Hin*dIII-*Bgl*II fragment corresponding to the region from exon 1′ to intron 7 of the murine *blk* gene (6) (long arm of homology) was cloned in the *Hpa*I site of pTV-0.

Generation of mice harboring the *blk* mutation. The *Cla*I-linearized DNA of the *blk* targeting construct (pTV-0/Blk) was transfected by electroporation into E14-1.1 cells followed by selection in the presence of G418 (300 μg/ml) and ganciclovir (2 μM) as described previously (12). The DNA of doubly resistant embryonic stem (ES) cells was digested with *Bam*HI and tested for the presence of the targeted *blk* allele by Southern blot analysis with the *Hin*dIII-*Bam*HI 1.5-kb DNA fragment of intron 9 as a probe (see Fig. 1A). This probe recognizes 8- and 4-kb DNA fragments of endogenous and mutated *blk* loci, respectively (see Fig. 1A). The presence of a single copy of the integrated targeting vector was confirmed by Southern blot analysis with the Neo^r gene as a probe. ES cell clones heterozygous for the *blk* mutation were injected into CB20 blastocysts, and the resulting chimeras were crossed to CB20 mice in order to identify the most efficient germ line-transmitting chimeric mice. The germ line-transmitting chimeras were crossed to 129/Sv mice, and heterozygous mice carrying the *blk* mutation were identified by Southern blot hybridization and intercrossed to produce homozygous offspring on the 129/Sv background. Age- and sex-matched 129/Sv and $blk^{-/-}$ mice were used in the experiments. Most of the experiments were done with 6- to 8-week-old mice. To facilitate the typing of $blk^{+/-}$ and $Blk^{-/-}$ mice, a PCR strategy was developed. The *blk* wild-type allele was specifically amplified with a primer located in exon 8 (5′ ATG TCA CCG GAA

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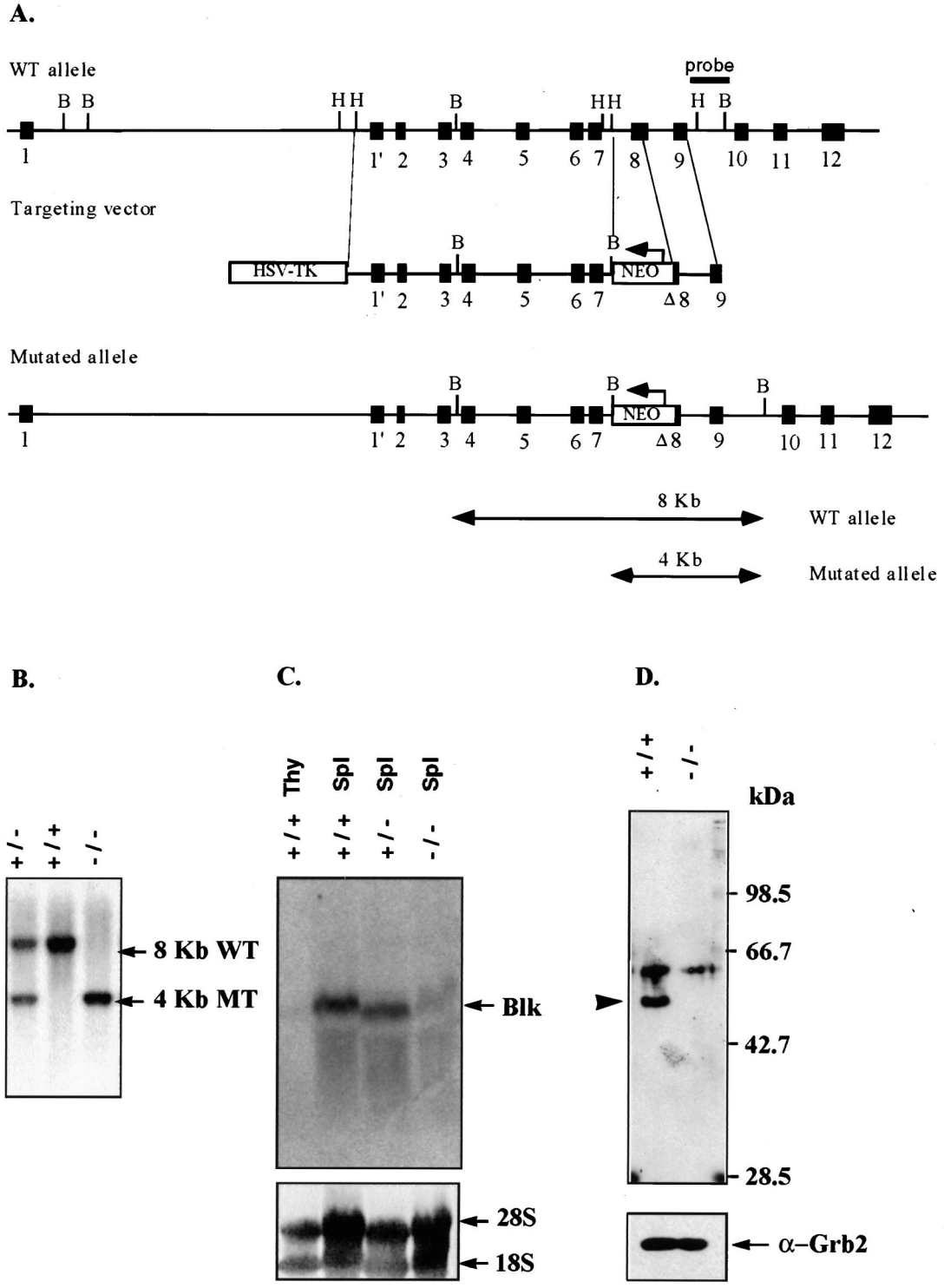

FIG. 1. Targeted disruption of the murine *blk* gene by homologous recombination. (A) Schematic representation of the *blk* genomic locus, with the targeting construct shown below. The arrows indicate the direction of transcription of the Neo^r and HSV TK genes. The positions of *blk* exons are shown as boxes. The indicated external probe recognizes 8 and 4 kb of the *Bam*HI-digested DNA of the wild-type and targeted *blk* genes, respectively. (B) Southern blot analysis of the *blk* mutation in mice. The tail DNA isolated from the wild-type 129/Sv, $blk^{+/-}$, and $blk^{-/-}$ mice was digested with *Bam*HI and analyzed as described above. Arrows indicate the position of the DNA fragments corresponding to the wild-type (8-kb) (WT) or targeted (4-kb) *blk* (MT) alleles. (C and D) Blk mRNA and protein expression in $blk^{-/-}$ mutant mice. Total RNA was prepared from lysates of splenic B cells and thymocytes of the wild-type 129/Sv, $blk^{+/-}$, and $blk^{-/-}$ mice. (C) *blk* RNA expression was analyzed by Northern blotting with a *blk* cDNA probe which contains the entire *blk* coding sequence (6). (D) Blk protein expression was analyzed by Western blotting of B-cell lysates with rabbit polyclonal antibody that recognizes the unique N-terminal domain of Blk plus the SH3 and SH2 domains. The position of Blk is marked by an arrowhead. Protein loading was controlled by immunoblot staining with anti-Grb2 antibody.

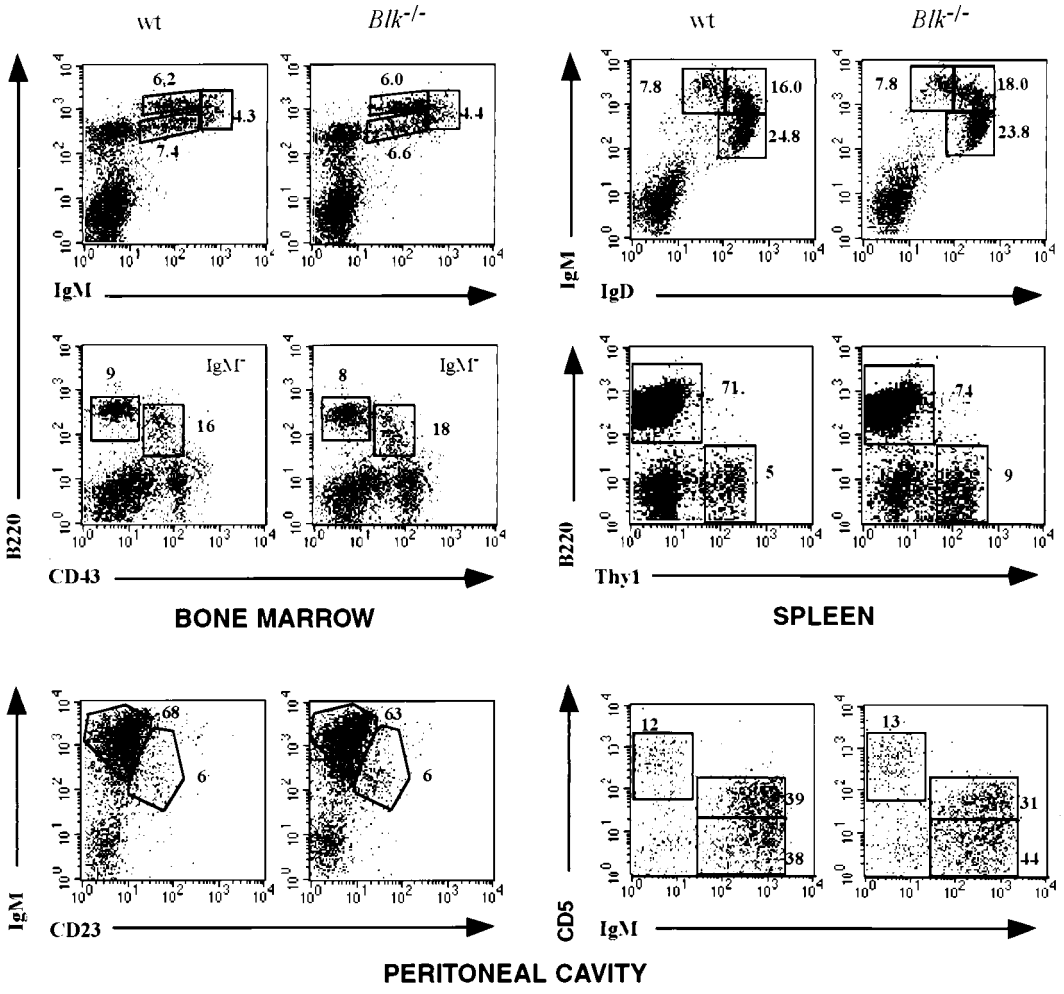

FIG. 2. Lymphocyte populations in Blk-deficient mice. Flow cytometric analysis of bone marrow cells, splenocytes, thymocytes, and peritoneal cavity cells in 8-week-old wild-type 129/Sv mice and Blk-deficient mice is shown. Numbers indicate the percentage of gated cellular subpopulations within the lymphocyte population.

GCT TTC C 3′; aa 270 to 275) and a reverse primer that hybridizes to a sequence in exon 9 (5′ A CCT GCT ACC TTC ATC GGT C 3′, aa 320 to 326). The *blk* mutant allele cannot be amplified with this pair of oligonucleotides since the sequence information of exon 8 encoding aa 252 to 284 is missing. The *blk* mutant allele was detected by using a primer complementary to a Neo^r gene sequence (5′ TAG CCG AAT AGC CTC TCC AC 3′; nucleotides 786 to 805) and the primer hybridizing to exon 9 described above. The annealing temperature was 60°C, and the Mg^{2+} concentration was 2 mM. The PCR products obtained were 1.2 and 1.5 kb for the wild-type and mutant *blk* alleles, respectively.

Cell staining and flow cytometry. Single-cell suspensions were prepared from different lymphoid organs and incubated for 10 min at 10^6 cells/20 μl on ice in staining buffer (phosphate-buffered saline [PBS] containing 0.5% bovine serum albumin [BSA] and 0.01% NaN_3) with optimal amounts of fluorescein isothiocyanate-, phycoerythrin-, or biotin-conjugated antibodies. The following monoclonal antibodies were purchased from Pharmingen (San Diego, Calif.): S7 (anti-CD43), B3B4 (anti-CD23), and Ly1 (anti-CD5). The following antibodies were prepared: RA3-6B2 (anti-B220), R33-24.12 (anti-IgM), 1.3-5 (anti-IgD), and Cfo-1 (anti-Thy1.2). Flow cytometric analysis was performed on a FACScan cytometer (Becton Dickinson & Co., Mountain View, Calif.).

Analysis of B-cell proliferation and upregulation of activation markers. Splenic B cells were purified by depletion of non-B cells on MACs columns (Miltenyi Biotec, Bergisch Gladbach, Germany) with anti-CD43 antibody coupled to magnetic beads (Miltenyi Biotec) as described previously (17). The purity of B cells was controlled by fluorescence-activated cell sorter analysis, and the preparations of B cells of 95% purity were used. B cells were stimulated with goat anti-IgM antibody (2.5 μg/ml) (Dianova, Hamburg, Germany), anti-CD40 antibody (0.6 μg/ml) (Pharmingen), and IL-4 (25 U/ml) (Genzyme Corp., Boston, Mass.). The analysis of cell proliferation and upregulation of activation markers was performed as described previously (3, 14).

Analysis of protein expression and tyrosine phosphorylation. For the analysis of protein expression, cells were lysed in lysis buffer (10% glycerol, 1% Triton X-100, 20 mM Tris-HCl [pH 8.0], 150 mM NaCl, 2 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10 μg of leupeptin per ml, 10 μg of aprotinin per ml). The lysates equivalent to 5×10^6 cells were loaded onto a sodium dodecyl sulfate (SDS)–10% polyacrylamide gel, and the separated proteins were electrotransferred to a Hybond nitrocellulose filter (Amersham) by semidry method. After being subjected to blocking with PBS–0.5% BSA–0.1% Tween 20, the filter was incubated first with a rabbit polyclonal antibody that recognizes the unique domain of Blk plus the SH3 and SH2 domains and then with a horseradish peroxidase-conjugated goat anti-rabbit IgG (Amersham) and developed with the enhanced chemiluminescence system (Amersham). RNA was analyzed by Northern blot analysis (20) using a blk cDNA probe (6). This probe (2,094 bp) contains the entire Blk coding sequence. For the analysis of tyrosine phosphorylation of whole-cell lysates and specific substrates downstream of Blk, purified B cells were suspended in RPMI supplemented with 2% fetal calf serum and stimulated with 20 μg of F(ab′)$_2$ fragment of goat anti-mouse IgM per ml for the indicated time (see Fig. 3) at 37°C. After centrifugation, cells were lysed in lysis buffer containing 1% Nonidet P-40. Whole-cell lysates corresponding to 5×10^5 cells were resolved by SDS-polyacrylamide gel electrophoresis (PAGE) (10% polyacrylamide). The rest of the lysates (representing 2.5×10^6 cells) were incubated with either anti-Syk (a generous gift from C. A. Lowell), anti-phospholipase C-γ2 (PLCγ2) (Santa Cruz, Santa Cruz, Calif.), or anti-Grb2 (Transduction Laboratories) antibodies for 1 h and then with protein A-Sepharose (Pharmacia) for 30 min. Sepharose beads were washed three times with lysis buffer and subjected to SDS-PAGE. The proteins were transferred onto a polyvinylidene difluoride membrane (Millipore, Bedford, Mass.), incubated with PY99 antiphosphotyrosine antibody (Santa Cruz), and detected with the Supersignal System (Pierce, Rockford, Ill.). For the analysis of protein tyrosine phosphorylation, purified B cells were suspended in serum-free RPMI 1640 for 1 h and then stimulated for

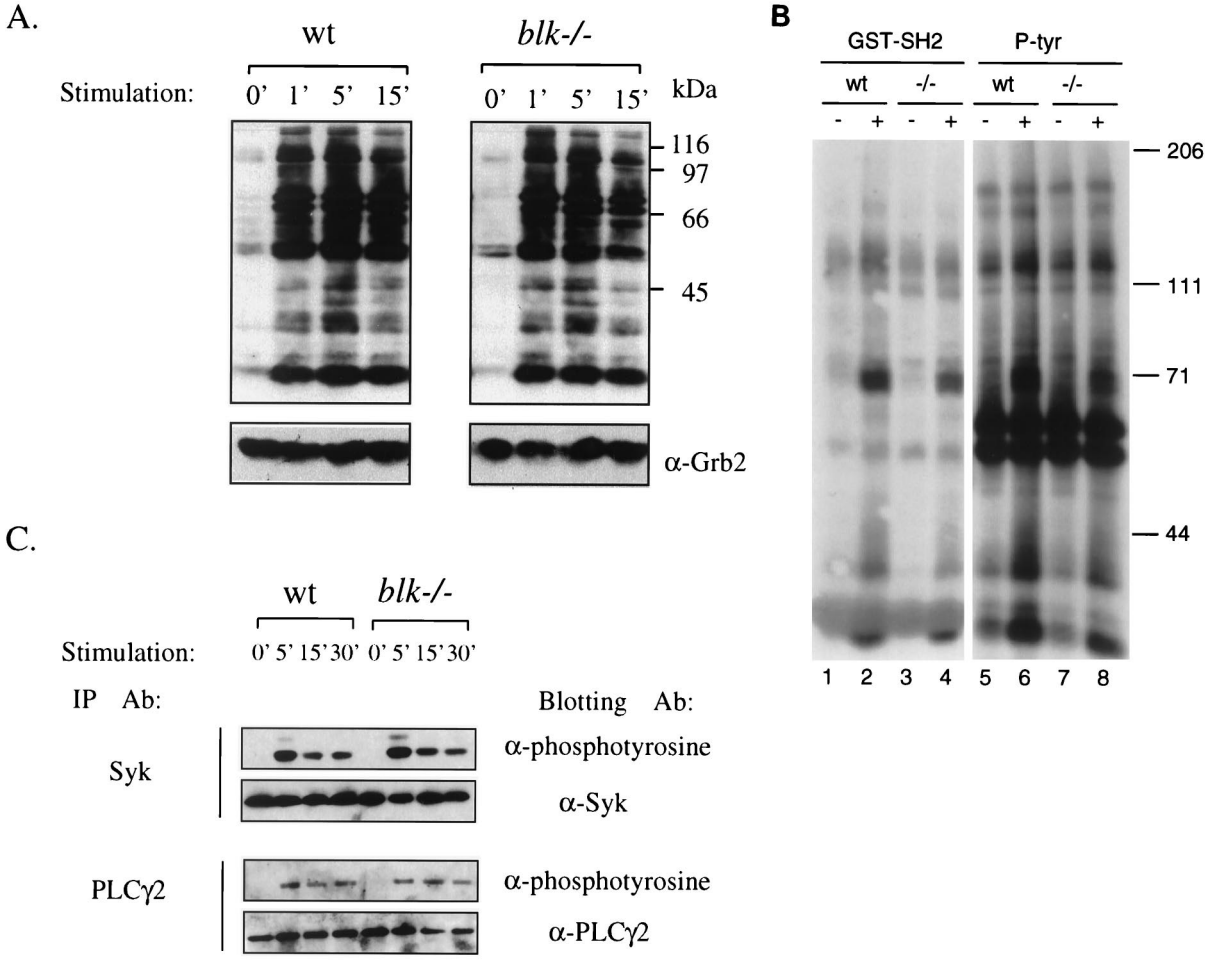

FIG. 3. Anti-IgM-induced protein tyrosine phosphorylation in B cells. Purified splenic B cells were stimulated with medium alone or goat anti-IgM at 37°C for the indicated periods, and cell lysates were prepared. (A) Whole-cell lysates were resolved on by SDS-PAGE and transferred to polyvinylidene difluoride membranes, and phosphorylation of the transferred proteins was determined by incubation of membranes with antiphosphotyrosine antibody PY99. (B) Cell lysates of unstimulated (lanes 1, 3, 5, and 7) or anti-IgM-treated (lanes 2, 4, 6, and 8) wild-type control (wt) and Blk-deficient (−/−) purified splenic B cells were precipitated either with the glutathione *S*-transferase–Blk SH2 domain fusion protein (lanes 1 to 4) or with the antiphosphotyrosine monoclonal antibody 4G10 (lanes 5 to 8). Precipitated proteins were fractionated by SDS-PAGE and transferred to nitrocellulose, and phosphotyrosine-containing proteins were detected by immunoblotting with the 4G10 antibody by using the enhanced chemiluminescence system. Positions of molecular mass markers and their apparent sizes (in kilodaltons) are indicated on the right. (C) Whole-cell lysates were immunoprecipitated with anti-Syk and anti-PLCγ2 antibodies, the immunoprecipitates were resolved by SDS-PAGE, and the phosphorylation of the immunoprecipitated proteins was analyzed by immunoblotting with antiphosphotyrosine antibody PY99. Equal protein loading was verified by stripping and reprobing the immunoblots with the indicated antibodies.

15 min at 37°C with 15 μg of goat anti-mouse IgM per ml at a density of 5×10^7 cells/ml. The cells were pelleted by centrifugation and then lysed in lysis buffer containing 1% Nonidet P-40. The cell lysate was clarified by centrifugation for 10 min at 12,000 × *g*. Aliquots of supernatants were incubated for 2 h with 20 μg of bead-immobilized glutathione *S*-transferase–Blk SH2 domain fusion protein or 10 μg of bead-immobilized antiphosphotyrosine monoclonal antibody 4G10. The beads were collected by centrifugation and washed four times with lysis buffer. The pellets were boiled in SDS-PAGE loading buffer, and the protein was fractionated by electrophoresis through an SDS–8% polyacrylamide gel. The protein was transferred to nitrocellulose. Phosphotyrosine-containing proteins were detected by immunoblotting with 4G10 antibody, and the membrane-bound antibody was detected by enhanced chemiluminescence.

Immune response to T-cell-independent and T-cell-dependent antigens. Wild-type 129/Sv and Blk-deficient mice were immunized intraperitoneally (i.p.) with 5 μg of the T-cell-independent antigen NP_{25}-Ficoll in PBS or 100 μg of the alum-precipitated T-cell-dependent antigen 4-hydroxy-3-nitrophenylacetyl–chicken γ-globulin conjugate (NP_{15}-CG) (three to five mice per group). For the analysis of secondary immune responses, the NP_{15}-CG-immunized mice were reimmunized i.p. 21 days after primary immunization. The concentration of NP-specific antibodies in serum at different time points were measured by an enzyme-linked immunosorbent assay. The assay was performed by coating plastic plates with NP_{15}-BSA (10 μg/ml), and serial serum dilutions were applied onto the plate. Bound antibodies were revealed by using biotinylated antibodies specific for a particular isotype as described previously (19).

RESULTS AND DISCUSSION

Generation of Blk-deficient mice. To inactivate the *blk* gene, E14-1.1 ES cells were transfected with the targeting construct shown in Fig. 1A. In this vector, the core portion of exon 8 of the *blk* gene is replaced by a neomycin resistance (Neo^r) gene, thereby disrupting the sequence encoding the essential part of the kinase domain of Blk. Separate clones of E14-1.1 ES cells which carried the disrupted *blk* gene were used to generate heterozygous ($blk^{+/-}$) and homozygous ($blk^{-/-}$) mutant mice (Fig. 1B). The *blk* mRNA expression levels in the purified splenic $blk^{-/-}$ B cells were below the detection limit of the Northern hybridization analysis (Fig. 1C). The RNA species carrying exons 1 to 4 of the *blk* gene could be detected by reverse transcription-PCR analysis of the RNA isolated from

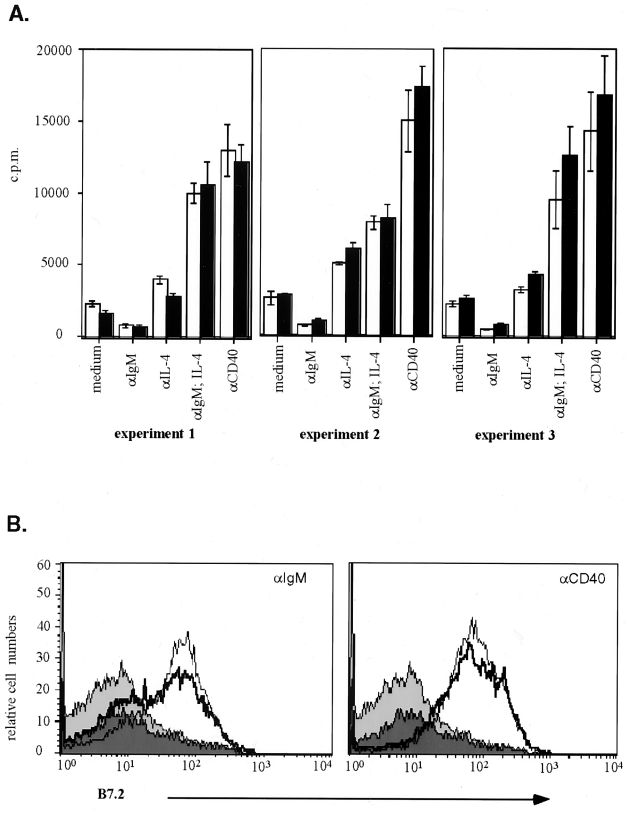

FIG. 4. B-cell activation in vitro. (A) Proliferative responses of B cells. The amount of [^{3}H]thymidine incorporated into the DNA of stimulated purified splenic B cells isolated from 129/Sv control (white bars) and Blk-deficient (black bars) mice is shown. All analyses were done in triplicate. (B) Upregulation of surface CD86 (B7.2). Histograms show the surface expression levels of CD86 (B7.2) on purified splenic B cells isolated from 129/Sv (thin line, light grey area) or Blk-deficient (thick line, dark grey area) mice. Cells were incubated with medium in the absence (filled area) or presence (line) of stimuli.

blk$^{-/-}$ B cells (data not shown). However, these RNAs were unable to give rise to a truncated Blk polypeptide(s) recognized by the anti-Blk antibody directed against the N-terminal portion of Blk (Fig. 1D). Furthermore, the truncated Blk proteins were not found in purified CD19$^+$ bone marrow B-lineage cells or in purified splenic B cells activated by anti-IgM antibody alone or in combination with IL-4 (or CD40 in combination with IL-4) (data not shown). Collectively, these data show that the targeted modification of the *blk* gene leads to Blk deficiency in *blk*$^{-/-}$ mice.

Development of B and T cells is unaltered in Blk-deficient mice. To analyze the potential influence of Blk deficiency on B-cell development and maturation, B-lineage cells from bone marrow, spleen, lymph nodes, and peritoneum of Blk-deficient mice and control 129/Sv mice were analyzed by flow cytometry. The total cell numbers and the frequency of B cells in spleen and lymph nodes were the same in Blk-deficient and control animals (data not shown). The bone marrow compartments of Blk-deficient and control mice were similar in terms of total cell number or the proportion of the pro-B cells (Ig$^-$ B220^{low} CD43$^+$), pre-B cells (Ig$^-$ B220^{low} CD43$^-$), immature B cells (IgM$^{lo/hi}$ B220^{high} CD43$^-$), and recirculating B cells (IgM^{lo} IgD^{hi} B220^{high} CD43$^-$). As in the 129/Sv control mice, about 95% of the splenic peripheral B cells expressed surface Ig containing κ chains (data not shown). The proportion of immature IgM^{hi} IgD^{lo} and mature IgM^{lo} IgD^{hi} B cells was the same in the spleen and lymph nodes of Blk-deficient and control mice (Fig. 2). Furthermore, the expression levels of surface proteins such as major histocompatibility complex class II, CD19, and CD23 were similar in wild-type and Blk-deficient splenic B cells (data not shown). Peritoneal B-1 (IgM^{hi} B220^{low} CD23) cells, including CD5-positive B-1a lymphocytes, were present at the same frequencies in the peritoneal cavities of control and Blk-deficient mice (Fig. 2). Collectively, these data demonstrate that development of B cells in the bone marrow and their maturation in the peripheral lymphoid organs are not dependent on Blk.

The exclusive expression of Blk in B cells has been challenged by the report on Blk expression in human thymocytes (10). Although the lack of Blk mRNA and protein expression in mouse thymocytes does not support these data (Fig. 1C), a possible effect of Blk deficiency on T-cell development was investigated. The thymuses of Blk-deficient and control mice were of equal size, and the ratios of CD4 and CD8 cells in the thymuses and spleens of Blk-deficient mice were the same as in 129/Sv control mice (data not shown). We also did not detect any difference in T-cell receptor αβ, CD3ε, heat-stable antigen, and CD69 expression in splenic T-cell and thymocyte subpopulations from control and Blk-deficient mice (data not shown).

Protein tyrosine phosphorylation. The role of Blk in BCR-induced signaling was addressed by the analysis of surface IgM-mediated tyrosine phosphorylation of intracellular proteins in purified splenic B cells. The patterns of phosphoproteins in whole-cell lysates of unstimulated and anti-IgM-treated 129/Sv control and Blk-deficient B cells were very similar (Fig. 3A). Since a deficiency of Blk could have specifically affected the phosphorylation of Blk-associated proteins, the phosphorylation of proteins which bind to the SH2 domain of Blk (Blk-SH2) was specifically analyzed. Similar to the proteins of whole-cell lysates, the phosphorylation of Blk-SH2-binding proteins was unaffected by the absence of Blk (Fig. 3B). Moreover, the anti-IgM-induced phosphorylation of known components of the BCR-dependent signaling chain such as Syk and PLCγ2 was similar in the wild-type and Blk-deficient splenic B cells (Fig. 3C). The lack of obvious changes in the pattern of the anti-IgM-induced protein tyrosine phosphorylation in the Blk-deficient B cells suggests a functional redundancy of Blk in BCR-induced B-cell activation. Indeed, antibody-mediated cross-linking of surface IgM on Blk-deficient cells led to upregulation of CD86 (B7.2) and major histocompatibility complex class II on the cell surface (data not shown) as well as to proliferation of mutant cells at levels similar to those of control cells (Fig. 4). The magnitudes of the proliferative responses of Blk-deficient and control splenic B cells to various amounts of anti-IgM were similar as well (data not shown). These data show that the Blk deficiency does not alter the threshold for anti-IgM-induced B-cell proliferation. The src-family PTKs are implicated in signal transduction mediated by B-cell-expressed surface receptor proteins such as CD38 and, to lesser extent, RP-105 (3). However, activation of Blk-deficient splenic B cells by anti-CD38 or anti-RP-105 is not impaired (3). Furthermore, proliferative responses of Blk-deficient cells to triggers of innate responses such as lipopolysaccharide or CG-rich oligonucleotides (11) are also unaltered (data not shown).

Blk-deficient mice respond efficiently to T-cell-dependent and independent antigens. To assess the response of *Blk*-deficient mice to environmental antigens, the concentrations of immunoglobulins of various isotypes in the sera of mutant mice were determined. Immunoglobulins of various isotypes were present in the sera of Blk-deficient mice at levels similar to those seen in control mice (Fig. 5A). To test whether Blk-deficient B cells are able to mount an antibody response upon

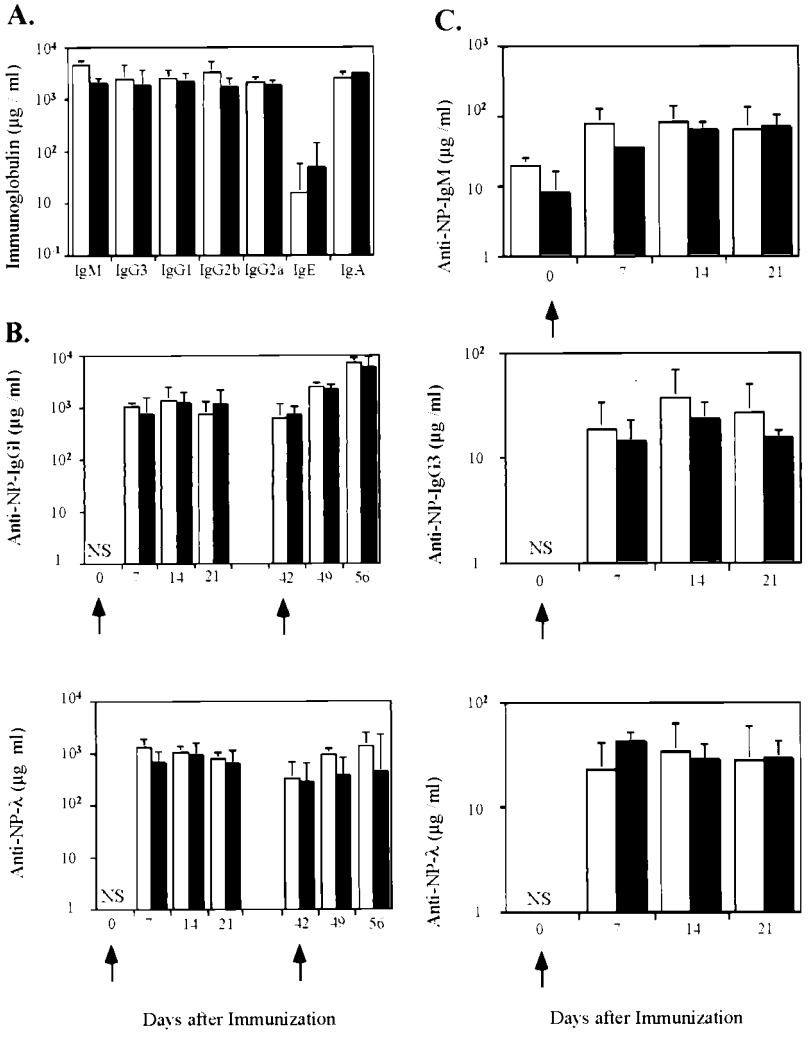

FIG. 5. Serum immunoglobulin isotypes in unimmunized and immunized Blk-deficient mice. (A) Serum immunoglobulin isotypes in unimmunized control (white bars) and Blk-deficient (black bars) mice. (B) NP-specific antibodies in sera of 129/Sv and Blk-deficient mice immunized with the T-cell-dependent antigen NP-CG. Concentrations of NP-specific IgG1 (upper panel) and λ-bearing (lower panel) antibodies in sera of 129/Sv (open bars) and Blk-deficient (black bars) mice were determined at different times after immunization with NP-CG (primary response). For the analysis of the secondary immune responses, mice of the same groups were reimmunized i.p. 21 days after the primary immunization and the titers of antibodies were determined at different times thereafter. (C) NP-specific antibodies in sera of 129/Sv (open bars) and Blk-deficient (black bars) mice immunized with NP-Ficoll. The titers of NP-specific IgM, IgG3, and λ-bearing antibodies were determined on days 7, 14, and 21 after immunization with NP-Ficoll. Geometric mean values and standard errors of the mean obtained from individual sera of three to five mice per group are shown. NS, not significant.

intentional immunization, Blk-deficient mice were immunized with the T-cell-dependent antigen NP-CG (8) and the T-cell-independent antigen NP-Ficoll (15). The concentration of hapten-binding antibodies was determined at different time points after immunization. In Blk-deficient mice, the primary response to NP, measured on days 7, 14, and 21 after immunization with NP-CG, was similar to that in control mice (Fig. 5B). Furthermore, secondary anti-hapten responses in Blk-deficient and control mice did not differ significantly (Fig. 5B). For the T cell-independent immunogen, both Blk-deficient and control mice mounted a humoral immune response at similar levels (Fig. 5C).

Concluding remarks. The experiments described here failed to reveal any defect in the immune system of Blk-deficient mice. Neither B-cell development nor B-cell responses in vitro and in vivo were altered by the lack of Blk. Although Blk is the only known B-cell-specific src-family PTK, our data suggest that Blk has no unique function in B-cell signaling and that other src-family PTKs expressed in Blk-deficient B cells can compensate for the lack of Blk. Our preliminary results on unaltered development and activation of Blk/Fyn doubly deficient B cells point to a key role of Lyn in src-family PTK-mediated B-cell functions in vivo. Analysis of Blk/Lyn doubly deficient and Blk/Fyn/Lyn triply deficient mice may elucidate the role of Blk and other src-family PTKs in B-cell signaling.

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G. Texido and I.-H. Su contributed equally to this work.

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