Role of Syk in B-cell development and antigen-receptor signaling

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Antigen receptors (BCRs) on developing B lymphocytes play two opposing roles—promoting survival of cells that may later bind a foreign antigen and inhibiting survival of cells that bind too strongly to self-antigens. It is not known how these opposing outcomes are signaled by BCRs on immature B cells. Here we analyze the effect of a null mutation in the Syk tyrosine kinase on maturing B cells displaying a transgene-encoded BCR that binds hen egg lysozyme (HEL). In the absence of HEL antigen, HEL-specific BCRs are expressed normally on the surface of Syk-deficient immature B-lineage cells, but this fails to promote maturation beyond the earliest stages of B-lineage commitment. Binding of HEL antigen, nevertheless, triggers phosphorylation of CD79α/β BCR subunits and modulation of receptors from the surface in Syk-deficient cells, but it cannot induce an intracellular calcium response. Continuous binding of low- or high-avidity forms of HEL, expressed as self-antigens, fails to restore the signal needed for maturation. Compared with the effects in the same system of null mutations in other BCR signaling elements, such as CD45 and Lyn kinase, these results indicate that Syk is essential for transmitting a signal that initiates the program of B-lymphocyte maturation.

The large repertoire of B lymphocytes that circulates in the blood and lymphoid tissues is formed from immature bone marrow precursors through a combination of positive and negative selection steps. These steps are determined by the clonally variable antigen receptors (BCRs) displayed on each B cell. Experiments in Ig-gene transgenic mice have shown that a single BCR specificity, displayed at the same stage of development, can promote or inhibit maturation and survival depending on the extent to which it chronically binds to self-antigens (1). How one receptor signals such opposing outcomes is unclear. Understanding this issue is likely to be important for treating B-cell autoimmunity, immunodeficiency, and lymphomas/leukemias of the B lineage.

The development and survival of B cells depends on the successful rearrangement of first heavy- and then light-chain Ig genes (2, 3). At the transition from the proB to preB cell stage, signals from the newly expressed preB cell receptor (preBCR), comprising the transmembrane form of IgM heavy chains paired with surrogate light chains λ5 and Vpreβ, suppress further heavy-chain gene rearrangement by products of the recombinase activating genes, *rag-1* and *rag-2*. Signals from the preBCR also initiate early preB cell clonal expansion and induce changes in proB cell markers, such as a decrease in the S7 epitope of CD43 and cessation of c-kit expression. Gene knockout and reconstitution experiments in transgenic mice establish that the preBCR signals for these events are transmitted via the membrane-spanning segment of the H chain and the cytoplasmic tails of noncovalently associated CD79α and CD79β chains (Igα and Igβ) (4–8).

Differentiation of preB cells into immature B cells is characterized by light-chain gene rearrangement and expression of H and L chains assembled into cell-surface IgM (9). Provided the BCR does not bind avidly to self-antigens, a program of maturation follows surface BCR expression. This program is characterized by expression of complement receptors that augment B-cell activation by foreign antigens, homing receptors such as L-selectin and Burkitt lymphoma receptor 1 that allow migration to peripheral lymphoid tissues after emigration from the bone marrow (10, 11), and a differentially spliced isotype of the BCR, IgD. Maturation and survival of B cells leaving the bone marrow continue to depend on signals from the BCR (12, 13) that require the *Src*-activating tyrosine phosphatase CD45, the immunoreceptor tyrosine activation motif of CD79α, and the tyrosine kinase Btk. CD45 deficiency diminishes but does not abolish BCR-induced calcium and extracellular regulated kinase activity, and the failure of surface BCRs to signal maturation and survival in the absence of CD45 can be restored by continued low-avidity BCR engagement with self-antigen (12).

Analysis of B-cell lymphomas and other transformed cell lines have identified the cytosolic protein tyrosine kinase p72^{Syk} (Syk) as essential for the B-cell antigen receptor to activate downstream signaling events such as intracellular calcium (14–16). In knockout mice, Syk-deficient (*syk*$-/-$) B-lineage cells in the bone marrow arrest at the CD43^{high} proB cell stage and few become B220^{high}CD43^{low} preB cells or surface IgM-bearing immature B cells (17, 18). In the spleen, scanty immature *syk*$-/-$ B cells have been reported in the red pulp where they can give rise to plasma cells, but they do not mature into IgM/IgD^{high} follicular B cells (19). Because few IgM-bearing cells are present in the bone marrow of *syk*$-/-$ mice and these have unknown specificities, it is difficult to determine if Syk, like CD45, is required for maturation after BCR expression. Here we investigate the role of Syk in BCR signaling and development *in vivo* by introducing a *syk* null allele into Ig-transgenic mice where the B cells carry a uniform, hen egg lysozyme (HEL)-binding BCR, Ig^{HEL}. Despite expressing HEL-specific BCRs, *syk*$-/-$ B cells accumulate at the earliest stage of maturation and fail to express complement receptors and other markers. Binding of low- or high-avidity forms of autoantigen has no effect on maturation of the *syk*$-/-$ Ig^{HEL} B cells but did induce IgM modulation in an exaggerated manner, indicating that antigen-receptor signaling is at least partially intact. Indeed, in primary B cells lacking Syk, BCR stimulation can still induce CD79α/β phosphorylation but cannot elicit a calcium flux.

Materials and Methods

Mice. C57BL/6 MD4 Ig^{HEL} transgenic mice were mated with heterozygous *syk*$+/-$ mice (18). The *syk* null allele was gener-

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Abbreviations: BCR, antigen receptor; HEL, hen egg lysozyme; Syk, p72^{Syk}; *syk*$-/-$, Syk-deficient; sHEL, soluble HEL; mHEL, membrane-bound HEL; Ig^{HEL}, anti-hen egg lysozyme Ig.

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ated previously by targeted disruption of the exon encoding for subdomain VI and confirmed *in vivo* by the absence of expressed Syk on Western blots (18). To minimize the effect of potentially modifying genes from 129/Sv, a series of breeding pairs were established with intercrossing *syk*+/− heterozygotes. Mice from two or more of these intercrosses were used in experiments to control for any effects of strain background. *Syk*+/− Ig^{HEL} progeny were mated with heterozygote nontransgenic partners in a series of intercrosses. MD4 encodes an anti-HEL Ig with both IgM and IgD isotypes (20). The C57BL/6 ML5 HEL-transgenic mice carry a transgene encoding HEL under the metallothionien promoter and contains HEL at a 1 nM level in serum (20); the C57BL/6 KLK HEL-transgenic mouse carries a transgene encoding a membrane-bound HEL under the H-2k promoter (21). Each of the transgenic mice is available from the Induced Mutant Repository of The Jackson Laboratory.

Screening Mutant and Transgenic Mice. A PCR assay was used to genotype Syk mutants by amplifying the neomycin-resistance gene in the targeted allele (18), and Ig^{HEL} and HEL transgenes were screened by PCR as described (22). Homozygous *syk*−/− fetuses were easily identifiable by the presence of extensive petechiae.

Fetal Liver Chimeras. Fetal liver cells were harvested at day 16.5 of gestation from embryos generated by intercrossing *syk*+/− Ig^{HEL} transgenic and *syk*+/− nontransgenic mice. Tissues were isolated as described (20), and 0.5–1.0 × 10^7 cells were injected into the lateral tail vein of recipients that had been lethally irradiated with two doses of 450 rads x-irradiation separated by 3 h. The animals received antibiotics (Polymixin B, 110 mg/liter and Neomycin, 1.1 g/liter) for the whole period of their reconstitution which was not less than 6 weeks or greater than 10 weeks. Chimeras of different genotypes were analyzed as complete cohorts; no differences were observed between mice that had been reconstituted for different periods.

Flow Cytometric Analysis. Three-color FACS analysis was performed on a FACScan with FACS desk software (Beckman Center Shared FACS facility) as described (20, 22). HEL binding was measured by incubating the cells with 200 ng/ml unlabeled HEL (Sigma) followed by biotinylated anti-HELMAb HyHEL9-TRIcolor (custom conjugation; Caltag, South San Francisco, CA).

IgM Modulation in Response to Antigen *in Vitro*. Bone marrow cells were isolated at room temperature, washed, and resuspended at a concentration of 1 × 10^6/ml in 5% FCS/RPMI medium 1640/10 mM Hepes. Cells were incubated in the presence or absence of HEL (1 μg/ml) at 37°C in 5% CO_2 for up to 8 h. Reactions were halted by the addition of an excess volume of ice-cold 0.5% FCS/PBS/0.01% sodium azide and analyzed by FACS.

Cell Lysates and Western Blot Analysis. Bone marrow cells were isolated at room temperature, stimulated with HEL (final concentration in media, 1 μg/ml), and immunoprecipitated with polyclonal CD79β and protein A, as described for splenocytes (23). Immunoprecipitated samples were resolved by 12% SDS/PAGE gels.

Calcium Analysis. Calcium analysis was conducted as described (24, 25). Cells were incubated with Indo-1AM (Molecular Probes) for 30 min at 37°C. mAbs to B220 (RA3–6B2-FITC or RA3–6B2-PE) were added to the incubation with Indo-1AM for the final 10 min.

Results

Expression of an IgM Receptor Does Not Rescue Development of Syk-Deficient Cells. To investigate whether provision of rearranged Ig H and L chain genes could overcome the developmental arrest in Syk-deficient B cells, mice heterozygous for a targeted disruption of the *syk* gene (*syk*+/−) were crossed with Ig^{HEL} transgenic mice carrying Ig heavy- and light-chain trans-

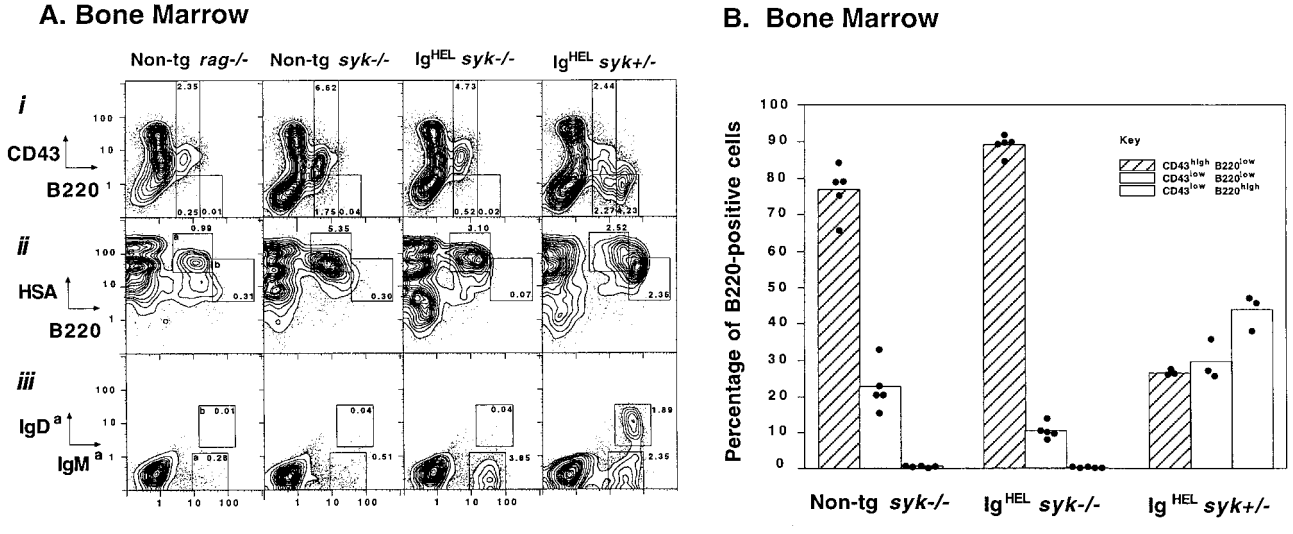


Fig. 1. Developmental arrest of Syk-deficient B cells in the bone marrow is not rescued by expression of rearranged Ig H + L transgenes encoding Ig^{HEL}. (*A*) B-cell development in bone marrow of nontransgenic *rag*−/−, nontransgenic *syk*−/−, and Ig^{HEL}-transgenic *syk*−/− and *syk*+/− chimeric mice, measured by two-color flow cytometry of cells stained for the indicated developmental markers. Windows in plots (*ii–iii*) are conventional for immature (*a*) and mature (*b*) B cells, and the frequency of cells are shown as a percentage of the total cells in the bone marrow. Plots of *syk*−/− and *syk*+/− bone marrows are each representative of six chimeras. (*B*) Histograms summarizing the percentage of B220-positive B-lineage cells in the bone marrow which are $CD43^{hi}B220^{lo}$ (Hardy A–C); $CD43^{lo}B220^{lo}$ (Hardy D–E); and $CD43^{lo}B220^{hi}$ (Hardy F). Bars represent arithmetic means and dots the percentages from individual chimeras. Mean numbers of B220 cells present in the bone marrow from one femur and one tibia of nontransgenic *syk*−/−, Ig^{HEL} *syk*−/− *and* Ig^{HEL} *syk*+/− *mice were* 20.10 × 10^4 (SD = 7.60); 14.42 × 10^4 (SD = 1.79); and 17.50 × 10^4 (SD = 3.90), respectively (SD = standard deviation × 10^{-4}).

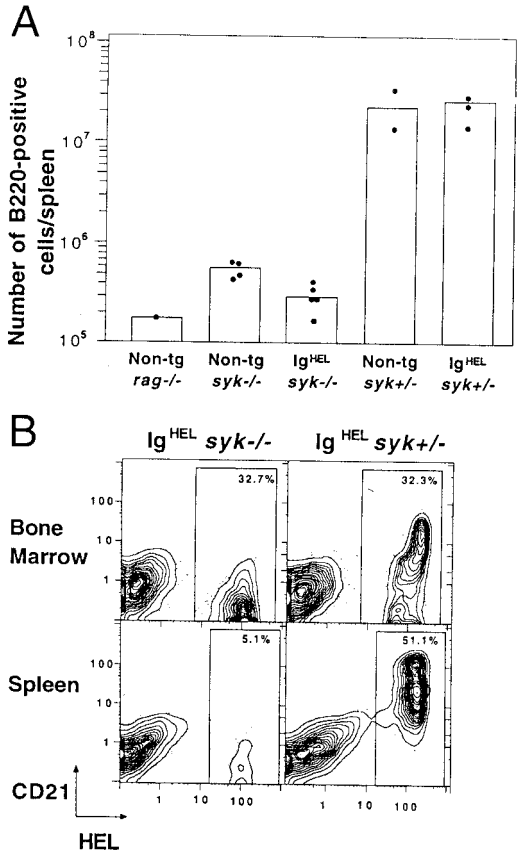
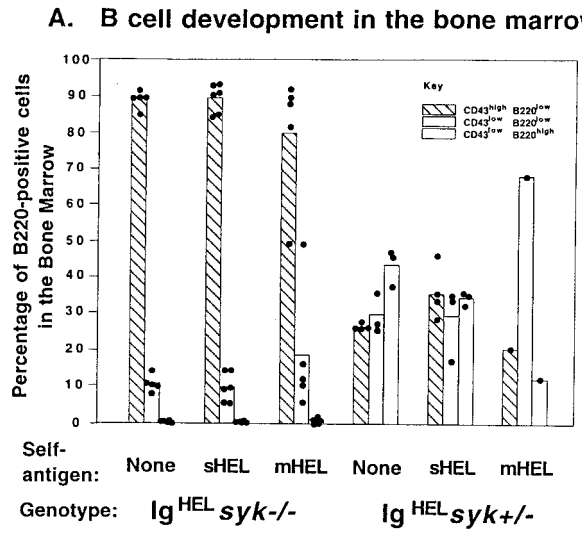


Fig. 2. The absence of *syk*−/− B cells in the blood and spleen is not corrected by expression of Ig^{HEL}. (*A*) Numbers of B220-positive cells in the spleen. Histograms show arithmetic means and dots represent individual mice which, with the exception of the *rag*−/− mouse, were all bone marrow chimeras. (*B*) Numbers and phenotype of B cells in the bone marrow and spleens of Ig^{HEL}-transgenic *syk*−/− and *syk*+/− chimeric mice, measured by two-color flow cytometry of cells stained for CD21 and HEL binding. Windows in plots show Ig^{HEL} transgenic B cells; the frequency of cells is shown as the percentage of the lymphocyte gate. Plots are each representative of six chimeras.



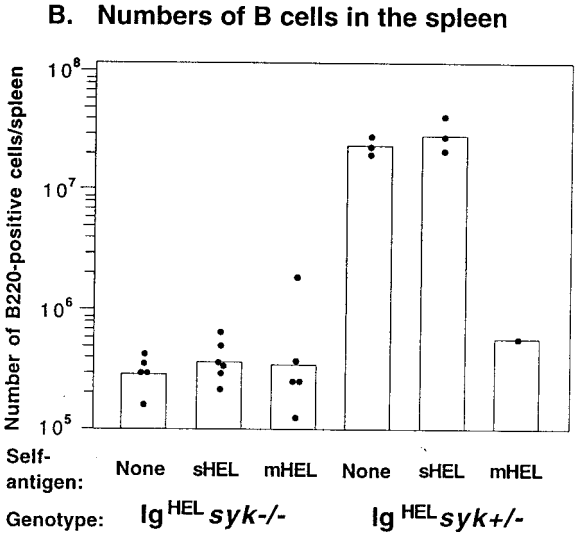


Fig. 3. Engagement of the BCR by self-antigens cannot rescue the development of Syk-deficient B cells. (*A*) Development of *syk*+/− and *syk*−/− Ig^{HEL} B cells in the absence of autoantigen (none) or the presence of sHEL or mHEL. Data were obtained and presented as in Fig. 1. The result from the single Ig^{HEL} *syk*+/− mHEL chimera is representative of published data (21, 22). (*B*). Numbers of B220-positive *syk*+/− and *syk*−/− Ig^{HEL} cells in the absence of autoantigen or the presence of sHEL or mHEL. Histograms show arithmetic means and dots represent individual chimeras.

genes encoding IgM^a and IgD^a specific for the well-defined protein antigen, HEL (20). To circumvent the perinatal lethality of the homozygous *syk*−/− mutation, which is caused by abnormal hemostasis, Syk-deficient hemopoietic stems cells were derived from fetal liver and used to reconstitute bone marrow radiation chimeras. Syk heterozygotes (Ig^{HEL} *syk*+/−) were phenotypically normal in a range of sensitive FACS and other tests.

Provision of the H- and L-chain transgenes restored surface IgM expression on the majority of *syk*−/− bone marrow B cells at levels equal to those on immature, IgD-negative B cells in the control mice (Fig. 1*A*, *iii*). Binding of HEL antigen to BCRs on nonmature B cells was also comparable in Ig^{HEL} *syk*−/− and *syk*+/− or +/+ controls (Fig. 4*A*).

Despite expressing transgenic surface IgM, other markers of development on Ig^{HEL} *syk*−/− B cells were similar to nontransgenic *syk*−/− cells (Fig. 1*A*). The proportion of B220+ cells in the bone marrow was not significantly different; the majority of these remained arrested at the $CD43^{hi}B220^{lo}$ stage, comparable to Hardy's fractions A–C (26) and the cells in *rag2*−/− mice (Fig. 1 *A* and *B*). In contrast to Ig^{HEL} *syk*+/− B-cell controls, the Ig^{HEL} *syk*−/− B cells showed no evidence of inducing markers of mature B cells. None of the cells were $B220^{hi}HSA^{lo}$ (Fig. 1*A*, *ii*); no IgD was expressed (Fig. 1*A*, *iii*); and no complement receptor CD21 was detected (Fig. 2*B*). These B cells remained large as judged by forward scatter (not shown).

In the absence of Syk, 100-fold fewer B220-positive cells were detected in the spleen (Fig. 2*A*), and these rare cells showed no evidence of maturation because they were $B220^{low}$, $CD21^{negative}$, and $IgD^{negative}$ as in the bone marrow (Fig. 2*B*).

Antigen-Induced Additional Signaling Cannot Compensate for the Absence of Syk. Continuous BCR engagement by soluble autoantigen (sHEL) is able to compensate for the signaling deficiency in CD45-deficient Ig^{HEL} B cells, allowing immature B cells to become fully mature and survive in the spleen and lymph nodes (12). To examine whether a similar compensation could occur in Syk-deficient cells, radiation chimeras were established with HEL-transgenic recipients. In the resulting animals, BCRs on

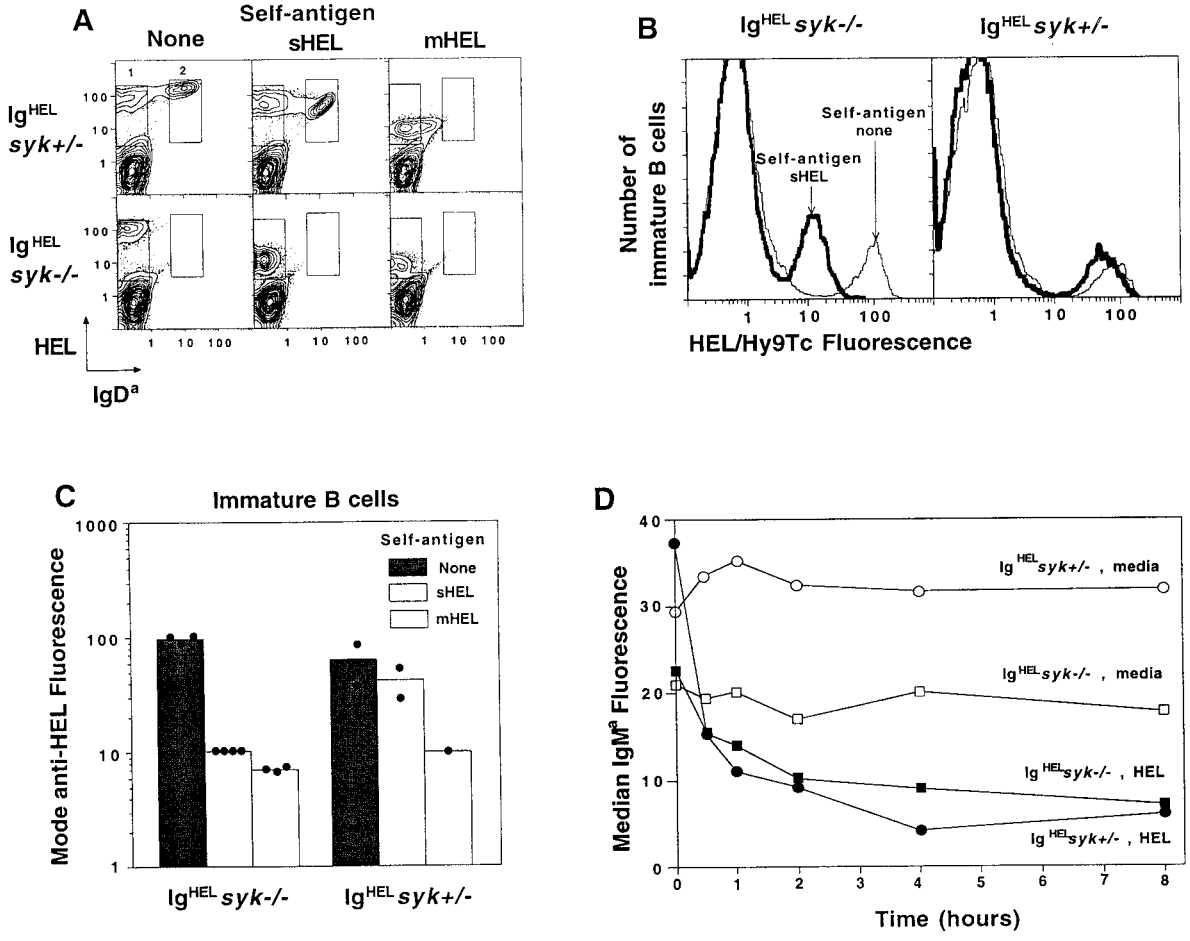


Fig. 4. Modulation of IgM in response to antigen does not depend on Syk. (*A*) Two-color flow cytometry of bone marrow cells from *syk*+/− and *syk*−/− Ig^{HEL} chimeric mice in the absence of autoantigen or the presence of sHEL or mHEL. The windows containing immature (1) and mature (2) B cells are indicated. Plots are representative of six chimeras. (*B*) Overlaid histograms of IgM expression (HEL/Hy9Tc fluorescence) on immature Ig^{HEL} B cells in the presence or absence of sHEL autoantigen. (*C*) Mode expression of HEL-binding BCRs on the surface of immature Ig^{HEL} B cells from *syk*−/− and *syk*+/− chimeric mice, developing in the absence of autoantigen or the presence of sHEL or mHEL. Histograms represent arithmetic means and dots individual animals. (*D*) Median IgM^a receptor levels on naive, immature $B220^{lo}$ Ig^{HEL} B cells from the bone marrow of *syk*−/− (squares) or *syk*+/− (circles) mice, after incubating *in vitro* for varying lengths of time with 1 μg/ml HEL (closed points) or with media alone (open points).

Ig^{HEL} B cells in the bone marrow were engaged by sHEL or multivalent membrane autoantigen (mHEL), but these stimuli had no effect on maturation markers or on the numbers of B cells in the spleen (Fig. 3 *A* and *B*).

The Syk-deficient Ig^{HEL} B-lineage cells nevertheless responded to BCR engagement by decreasing the numbers of BCRs displayed on the cell surface (Fig. 4 *A–D*). In the bone marrow of mice expressing sHEL or mHEL, *syk*−/− Ig^{HEL} B cells decreased their surface IgM levels by 10-fold relative to the levels present in the absence of antigen (Fig. 4 *B* and *C*). The occurrence of this response to sHEL is surprising, because the concentration and avidity of sHEL is normally insufficient to trigger this response in immature Ig^{HEL} B cells (gated IgD-negative *syk*+/− cells in Fig. 4 *B* and *C*), except when BCR signaling is exaggerated by defects in SH_2 domain hemopoietic phosphotase 1 recruitment (23, 25). *In vitro* modulation of IgM on *syk*−/− and *syk*+/+ Ig^{HEL} B cells in response to HEL was equivalent (Fig. 4*D*). These data indicate that the signals for antigen-induced modulation do not require Syk and may be exaggerated in its absence.

Antigen-Induced Phosphorylation of CD79α and CD79β Is Not Transmitted to Intracellular Calcium in the Absence of Syk. The modulation of IgM in response to autoantigen suggested that BCR signaling in the absence of Syk was not completely abolished. To examine this further, Syk-deficient Ig^{HEL} B-lineage cells were stimulated *in vitro* and examined for selected early signaling events that normally accompany activation. Antigen-induced phosphorylation of CD79α and CD79β did not require the activity of Syk, although the amount of tyrosine phosphorylation was reduced (Fig. 5*A*). In contrast, no elevation of intracellular calcium could be detected in the Ig^{HEL} *syk*−/− cells in response to sHEL antigen or polyvalent anti-IgM antibodies (Fig. 5*B*). These stimuli nevertheless evoked calcium responses in *syk*+/− $B220^{lo}$ Ig^{HEL} immature B cells from the bone marrows of control chimeras.

Discussion

B-cell maturation and survival fails if there is too little or too much signaling by the BCR (1), making it difficult to assign specific *in vivo* roles for different signaling components. For example, deficiency of CD45 or Lyn both result in fewer mature B cells being formed (12, 23), but for opposite reasons. Failure of maturation because of CD45 deficiency reflects a reduction in BCR signaling that can be rescued by continuous BCR engagement with a weak self-antigen agonist (12). In contrast, poor maturation caused by Lyn deficiency reflects exaggeration of

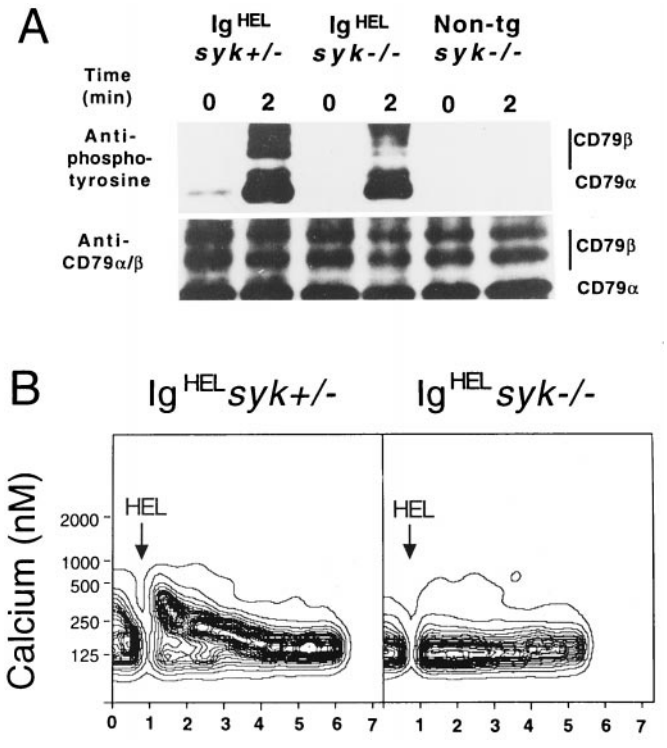


Fig. 5. Syk is not required for antigen-induced phosphorylation of CD79α and CD79β but is essential for a calcium response. (*A*) Western blot of immunoprecitated CD79β from lysates of *syk*$-/-$ and *syk*$+/-$ Ig^{HEL} and *syk*$-/-$ nontransgenic bone marrow cells. Cells were stimulated with HEL (1 μg/ml) for 2 min or left unstimulated. Panels show induced tyrosine phosphorylation by probing the immunoprecipitates for antiphosphotyrosine (4G10), relative to the total immunoprecipitated protein measured by reprobing the same blot with antisera to CD79α and β. Results are representative of three experiments. (*B*) Flow cytometry of indo-1-loaded cells was used to monitor intracellular calcium levels as a function of time after stimulation of Ig^{HEL} *syk*$-/-$ and *syk*$+/-$ B cells from bone marrow. All measurements were gated on immature, B220^{lo} cells. Arrows indicate time of addition of HEL antigen (1 μg/ml). Equivalent results were obtained from three separate experiments.

BCR signaling and the defect in forming mature B cells is made more severe by the same weak BCR agonist (23). By using the same analytical system, the data here indicate that failure of B-cell maturation caused by Syk deficiency occurs at an earlier developmental stage and reflects an essential role for the enzyme in transmitting signals from phosphorylated BCRs to intracellular calcium and to a set of transcriptional and migratory responses.

Six distinct processes help form the recirculating B-cell repertoire and have been shown to be coordinated by the BCR (2, 3), but only some of these appear to require Syk. The earliest of these is the proliferation of progenitor B cells, identified as large S7^{hi} B220^{low} cells in the bone marrow. Whereas a deficiency of IL-7, its receptor, or downstream janus kinase 3 results in a failure of B-cell formation at this stage, Syk deficiency causes no decrease in the number of large S7^{hi} B220^{lo} cells (Fig. 1). Instead, the frequency of these cells is increased markedly, presumably because they do not progress to more mature stages (Fig. 1*B*), although the possibility of compensatory proliferation cannot be ruled out.

Exit from the cell cycle, in contrast, to form small S7^{low} B220^{low} immature cells absolutely depends on Syk. This effect is known to depend on membrane IgM heavy chains and surrogate light chains, and it accounts for the suppressive effect of rearranged IgM transgenes on lymphomagenesis in mice with a constitutively expressed c-*myc* gene (27). The Ig^{HEL} BCR is active in transmitting this signal when Syk is intact, because the failure to form small S7^{lo} B220^{lo} immature cells in *rag2*$-/-$ mice is fully corrected by the Ig^{HEL} transgenes (J. Cyster and C.C.G., unpublished data). BCR-induced cessation of H-chain gene rearrangement (to bring about H-chain allelic exclusion) occurs at approximately the same stage and also depends on Syk (A.C. and T.P., unpublished data).

Once a complete IgM BCR is displayed on the surface of an immature B cell, this is normally followed by a progressive increase in cell-surface expression of CD45, IgD, complement receptors, Burkitt lymphoma receptor 1, and L-selectin homing receptors. Expression of these molecules, caused at least in part by transcriptional changes (e.g., IgD), facilitates B-cell activation by foreign antigens in spleen and lymph nodes. Signals from the BCR have been shown to actively inhibit this maturation program, in response to continued BCR engagement by high-avidity autoantigens (22) or low-avidity self-antigens when BCR signaling is exaggerated by mutations that interfere with SH_2 domain hemopoietic phosphotase 1 recruitment to the BCR complex (23, 25). The data here establish that Syk is absolutely required for this maturational program to begin. Syk deficiency prevented expression of even the low levels of IgD or complement receptors that normally can be detected on bone marrow cells that have begun to mature or on transitional cells that usually are detected in the spleen. In contrast, these gene products are expressed at low-medium levels in CD45-deficient cells or self-reactive cells where maturation is actively inhibited. This absolute defect in maturation-gene expression could not be compensated by any amount of BCR engagement (Fig. 3), in contrast to CD45 deficiency. Nevertheless, the number of immature B-lineage cells was slightly increased by Syk deficiency (Fig. 1), which implies that the Syk-dependent signal is needed in the bone marrow for the transcriptional response itself rather than for survival of cells that have begun transcribing IgD and other maturational markers.

Despite normal or slightly increased numbers of IgM expressing proB/immature B cells in *syk*$-/-$ bone marrow, the number of B cells in the spleen was reduced 100-fold. This decrease in peripheral B-cell number is much greater than occurs in the spleens of mice expressing membrane HEL self-antigen, where the numbers are only decreased 5- to 10-fold (22). This result suggests that Syk is required for migration out of the bone marrow stroma and into the bloodstream, either directly or as part of the maturation program discussed above. The few cells present in the spleen may have arisen as part of the extramedullary hemopoiesis that occurs in the spleen of mice. Active migration of thymocytes into the blood has been indicated previously based on a similar block in T-cell export caused by expression of the pertussis toxin (28). Like the maturation program, no amount of BCR engagement could correct for the defect in *syk*$-/-$ cell appearance or accumulation in the periphery.

Our findings are similar to those recently described with the 3–83 $\mu\delta$ anti-H2-K$^{k/d}$ transgene (19) in that we have shown the absence of mature cells in Syk-deficient Ig^{HEL} B cells, but they differ in that we have not shown that the transgene can rescue any significant progression to the CD43^{neg} immature B-cell stage. The transgene does not increase the low proportion of CD43^{neg} B cells in the bone marrow of nontransgenic *syk*$-/-$ mice or the number of IgM-expressing B cells in the spleen. One chief difference is that Turner *et al.* (19) concluded that Syk is needed relatively late in B-cell maturation, for positive selection by antigen or unknown BCR ligands after immature B cells have reached the spleen. In contrast, the lack of maturation in bone marrow and the rarity of cells reaching the spleen (above) lead us to conclude that Syk is needed in the bone marrow for the BCR, in the absence of any selecting ligand, to trigger expression

of mature B-cell genes, and possibly for a program that allows normal emigration to the secondary lymphoid tissues. It is interesting to speculate that the more extreme phenotype observed here might reflect differences in the V-regions or specificity of the BCR transgenes used in the two studies. Compared with the IgHEL *syk*−/− CD43^{lo} cells were formed in nontransgenic *syk*−/− bone marrow (Fig. 1). Thus, it is possible that a small fraction of BCRs, such as the 3–83 Ig-transgene, can promote maturation without Syk. The majority of BCRs appear not to allow maturation beyond the B220^{lo} CD43^{hi} stage, because most B-lineage cells are arrested at this stage in nontransgenic syk−/− bone marrow. Other possible variables are polymorphisms in modifier genes caused by different mouse strain backgrounds, and different degrees to which the targeted mutations result in a Syk-null state.

The antigen-induced phosphorylation of CD79α and β and the modulation of IgM in response to antigen *in vivo* and *in vitro* demonstrate that not all elements of B-cell signaling depend on Syk. These data suggest that CD79α/β phosphorylation is likely to be initiated by *src* kinases, such as Lyn, Lck, Fyn, and Blk, and lead to subsequent binding and activation of Syk. In the DT40 chicken B-cell line, BCR-induced activation of Syk clearly depends on the activity of Lyn, which is the single dominant *src* kinase in these cells (29, 30). The increased IgM modulation in response to self-antigen may be caused by the abnormal developmental stage of the pro/immature *syk*−/− B cells, but it is also possible that Syk-independent pathways are exaggerated by changes in the competition between kinases for the immunoreceptor tyrosine activation motif binding sites on CD79α and β. Modulation of IgM is a graded response to BCR signaling. It is proportional to receptor occupancy and antigen valency (31). It is exaggerated by signal-enhancing mutations in Lyn (23), SH_2 domain hemopoietic phosphotase 1 (25), and CD19 (32), and it is diminished by signal-lowering mutations in CD45 (12). The biochemical nature of this response is unknown, although it is caused by inhibition of BCR transport from the endoplasmic reticulum to the Golgi apparatus (33).

Collectively, the data here define the *in vivo* role for Syk in transmitting signals from the BCR to calcium and gene expression responses that underpin B-cell migration and maturation. It will be important in the future to define the downstream targets of Syk in promoting these responses, and explore additional roles for Syk in mature B cells by engineering conditional mutations or pharmacological inhibition.

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