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Genetic Evidence for Lyn as a Negative Regulator of IL-4 Signaling

Michelle L. Janas,* Philip Hodgkin,†‡ Margaret Hibbs,§ and David Tarlinton²*§

IL-4 has multiple effects on B lymphocytes, many of which are concentration dependent. This is particularly so for Ig isotype switching, where different thresholds of IL-4 stimulation are needed to induce switching from IgM to either IgG1 or IgE. In this report we describe a critical role for the tyrosine kinase Lyn in setting IL-4 signaling thresholds in mouse B lymphocytes. Upon CD40 ligand stimulation of lyn−/− B cells, 10-fold less IL-4 was required to induce switching from IgM to IgG1 and IgE and an increased proportion of B cells isotype switched at each IL-4 concentration. These in vitro results correlate with the in vivo findings that in lyn−/− mice, IgG1 Ab-forming cells develop prematurely in ontogeny and that adult lyn−/− mice have an abnormally high proportion of IgG1-expressing B cells in their spleens. Adult lyn−/− mice also have significantly higher levels of IgE in their serum. These results identify Lyn as a molecule involved in modulating the IL-4 signal in B cells and provide insights into its regulation and how a B cell signaling imbalance may contribute to atopy. The Journal of Immunology, 1999, 163: 4192–4198.

Interleukin-4, a cytokine produced by Th cells, basophils, and mast cells, has multiple effects on hemopoietic cells (1). Exposure to IL-4 affects B lymphocytes in three ways. First, IL-4 inhibits apoptosis in B cells, possibly by increasing the expression of the antiapoptotic molecule BCL-2 (2). Second, although IL-4 does not induce cell division alone, B cell proliferation is enhanced by the synergistic interaction of IL-4 and mitogens, such as LPS and F(ab')2 anti-Ig (3). IL-4 enhances mitogen-induced B cell proliferation through its ability to increase cell survival, allowing a greater proportion of B cells to enter the cell cycle, and by directly effecting proliferation (4). Third, IL-4 promotes Ig class switching from IgM and IgD on naive B cells to IgG1 and IgE (1). Secreted IgE is important in resolving parasite infections (1) and in causing certain hypersensitivity reactions (5).

IL-4 inhibition in B cells is mediated by a specific receptor, a heterodimer consisting of α and γ polypeptide chains (7). Upon IL-4 binding, various signal transduction molecules including the Janus activation kinases (8, 9) and the transcription factor STAT-6 (10), are recruited to the receptor and phosphorylated. Phosphorylated STAT-6 dimerizes, enters the nucleus, and binds to IL-4-responsive elements, thereby initiating transcription of downstream genes (10). IL-4-responsive elements are found in the promoters of IL-4-inducible genes such as CD23 (11), Cγ1 (12), and Cε (13). Indeed, transcription of germline Cγ1 and Cε is a prerequisite for switching to these isotypes (14). Although little is known about the negative regulation of IL-4 signaling, it is thought to be mediated by the phosphatase SHP-1 (15). B cells from moth-eaten viable (me⁺) mice, which have a mutation in the gene encoding SHP-1 such that the protein retains only 10% of wild-type catalytic activity (16), show enhanced activation of the transcription factor STAT-6 upon IL-4 stimulation (15). Through binding to the IL-4R, activated SHP-1 dampens or suppresses IL-4 signaling (15). For SHP-1 to be recruited to the IL-4R, its binding site needs to be phosphorylated. The kinase responsible for this phosphorylation of the IL-4R and, consequently, for initiating the subsequent negative regulation of the IL-4 signal is currently undefined.

The src-related tyrosine kinase Lyn targets a number of cell surface receptors for phosphorylation, including the B cell receptor (BCR), CD22, and FcγRIIb (17). At the BCR, Lyn phosphorylates tyrosines within the Igα-Igβ heterodimer, facilitating recruitment of the kinase Syk and subsequent initiation of positive downstream signaling events (17). This function of Lyn, however, appears to be redundant, because positive signals are transduced through the BCR in the absence of Lyn (17). This is presumably due to the ability of other src family members to associate with the BCR (18). A unique role for Lyn in B cells is the initiation of negative signaling cascades upon Ag stimulation. Upon BCR cross-linking, Lyn phosphorylates CD22 and thereby induces recruitment of the phosphatase SHP-1, which suppresses signals emanating from the BCR (19). In B cells, Lyn also phosphorylates the IgG receptor FcγRIIb (20), which negatively regulates B cell proliferation (17). As for CD22, the inhibitory function of FcγRIIb is mediated by the recruitment of a phosphatase, in this case SHIP (19). Lyn knockout

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mice have several immunological abnormalities, including a 10-fold increase in both IgM serum titers and the frequency of IgM Ab-forming cells (AFC), the production of autoantibodies and a deficiency in mature B cells (21, 22). This phenotype resembles that of the CD22 knockout (23) and mec-2 mice (24) and exemplifies the role of Lyn in initiating this particular negative signaling pathway.

The study reported here reveals a previously unsuspected role for Lyn as a negative regulator of IL-4 signaling in B cells. Mitogen-stimulated B cells from lyn−/− mice are hyper-responsive to IgG isotype switching induced by IL-4. As a consequence of this, adult lyn−/− mice have an increased frequency of IgG1 splenic B cells and a 10-fold increase in serum IgE. Our results show that in the absence of Lyn the threshold of IL-4 signaling is diminished, resulting in IL-4 hyper-responsive B cells. We hypothesize that Lyn acts by recruiting a phosphatase, possibly SHP-1, to the IL-4R.

**Materials and Methods**

**Mice**

The generation and characterization of lyn−/− mice have been described (21). Mice were originally a C57BL/6 × 129Sv intercross before an 11th generation. C57BL/6 backcross line was established. Lyn−/− mice were used as controls. Hemizygous transgenic mice of the Lyn−/− × lyn−/− background (C6 Diagnostics, Germantown, WI) were provided by Dr. A. W. Harris (The Walter and Eliza Hall Institute, Melbourne, Australia). Therefore these mice were crossed with lyn−/− mice, and the F1 mice were intercrossed to select for lyn−/− mice. Ly5.1 congenic C57BL/6 mice were used to distinguish lyn−/− mice from lyn−/− cell in in vitro coculture experiments.

**ELISPOT and ELISA assays**

IgM and IgG1 AFCs were detected by ELISPOT as previously described (21). Brieﬂy, cell culture-based assays (Millipore, Bedford, MA) were utilized. IgM was detected using a sheep anti-mouse IgM antibody (Nordic Immunology Associates, Birmingham, AL). AFC were stained with a mixture of Abs comprising PE conjugates of anti-CD40 (clone A20.1.7, Southern Biotechnology Association) and not saturating. PCR products were run through a 1.5% agarose gel before transfer to nylon membrane (Zybrex, Bio-Rad, Hercules, CA). The blot was hybridized with 32P end-labeled internal oligonucleotide probes for β-actin (Clontech) and IL-4R of the sequence 5'-(CCACGTTTAGG3'-3') and CAGCTGGAAGTGTGA 5'-(CCACGTGTTAGG3'-3'), respectively, in an 500-bp product. DNA was amplified for β-actin using oligonucleotides from Clontech (Palo Alto, CA). For amplification of the IL-4R a 25 cycles were used, which was previously determined to be in the linear range for product amplification and not saturating. PCR products were run through a 1.5% agarose gel before transfer to nylon membrane (Zybrex, Bio-Rad, Hercules, CA). The blot was hybridized with 32P end-labeled internal oligonucleotide probes for β-actin (Clontech) and IL-4R of the sequence 5'-(CCACGTTTAGG3'-3'). The results were visualized and quantified using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

**Results and Discussion**

Investigating the differentiation potential of lyn−/− B cells led us to test in the in vitro response of these cells to costimulation with CD40L (CD40/CD154) and IL-4. These experiments were performed in such a way that the extent of both B cell division and isotype switching could be quantified (26). IL-4-induced isotype switching to both IgG1 and IgE is known to be linked to cell division number with the proportion of switched cells achieved at any division, being related to the IL-4 concentration (27). High density B cells were labeled with the intracellular dye CFSE, which allows up to eight divisions to be counted by sequential halving of fluorescence (28). In this way the effects of IL-4 on B cell proliferation and switching were explored separately using FACS analysis to calculate the percentage of cells that have iso- type switched within any specific division cycle. Novel lyn−/− mice showed division-linked isotype switching to IgG1 over a range of IL-4 concentrations, starting at 1 U/ml. This is apparent in contour plots (Fig. 1A) and as the percentage of cells within each division that have switched (Fig. 1B). No wild-type B cells switched to IgG1 at 1 U/ml of IL-4 over eight cell divisions within 5 days. Control B cells commence switching to IgG1 at 10 U/ml IL-4, with the level increased at 100 U/ml. Thus, in contrast to control B cells, lyn−/− B cells switch to IgG1 earlier divisions and have a higher proportion of switched cells when a plateau is reached at the later divisions.

Analysis switching to IgE gave similar results (Fig. 2). Both lyn−/− and control B cells only initiated switching to IgE after seven cell divisions; however, at both 10 and 100 U/ml IL-4, the percentage of lyn−/− B cells expressing IgE was increased compared with the control value. These results were not due to the

**Immunostaining and flow cytometric analysis**

Cells harvested from cell culture were stained with PE-conjugated RA3-6B2 (CD45R, otherwise known as B220; Pharmingen, San Diego, CA) and Texas Red-conjugated goat anti-mouse IgG1 (Southern Biotechnology Associates) or biotinylated anti-IgE Ab R1E4, revealed with PE-streptavidin (Caltag, San Francisco, CA), along with propidium iodide for dead cell exclusion and analyzed a FACStarPlus (Becton Dickinson, San Jose, CA). Splenic IgG1 expressing B cells were identified as IgM−/− IgG1+CD45R+B and IgG1−. After RBC removal, single-cell suspensions were stained with a mixture of Abs comprising PE conjugates of 33-12 (anti-IgM) and 11-26C (anti-IgD, Southern Biotechnology Associates), fluorescein-conjugated RA3-6B2 (CD45R), and Texas Red-conjugated goat anti-mouse IgG1 (Southern Biotechnology Associates). Analysis was performed on a FACStarPlus. Ly5.1 C57BL/6 cells were identified using a fluorescein conjugate of the mAb A20.1.7.

**Quantification of IL-4R expression**

Splenic B cells were purified by FACS sorting, and 2.5 × 106 cells were cultured with CD40L and IL-4 for 24 h (described above) before RNA extraction using the RNasea Mini Kit (Quagen, Melbourne, Australia). For cells that were unstimulated, including the positive control cell line CTL-L2, RNA extraction from 2.5 × 106 cells immediately followed purification. One-tenth of the RNA product was converted to cDNA using Superscript II reverse transcriptase (Life Technologies, Grand Island, NY) in a mix containing random hexamer primers (Pharmacia, Piscataway, NJ) for 30 min at 37°C. The extracellular domain of the IL-4Rα was amplified by PCR from 1/50th of the cDNA using the oligonucleotides 5'-(ATCCCGACT TTACGTTGATGTTG3'-3') (forward) and 5'-(CGCCTGGAAGTGTGA CCACGTGTTAGG3'-3') (reverse), resulting in an ~550-bp product. cDNA was amplified for β-actin using oligonucleotides from Clontech (Palo Alto, CA). For amplification of the IL-4Rα and β-actin, 25 cycles were used, which was previously determined to be in the linear range for product amplification and not saturating. PCR products were run through a 1.5% agarose gel before transfer to nylon membrane (Zybrex, Bio-Rad, Hercules, CA). The blot was hybridized with 32P end-labeled internal oligonucleotide probes for β-actin (Clontech) and IL-4R of the sequence 5'-(CCACGTTTAGG3'-3'). The results were visualized and quantified using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).
differential death of isotype-switched B cells, because cell numbers in both cultures remained comparable throughout the experiment (data not shown). These results show that Lyn is involved in setting both the threshold of IL-4 responsiveness and the strength of that signal, at least where this is reflected in isotype switch.

Besides having elevated levels of IgM, there are several other immunological abnormalities in lyn−/− mice (21, 22). It could be

![FIGURE 1](image1.png)  
**FIGURE 1.** Isotype switching to IgG1 in response to IL-4 stimulation. A, Two-dimensional contour plots of CFSE intensity vs IgG1 expression in lyn+/+ (left panel) and lyn−/− B cells (right panel) stimulated with CD40L and IL-4 at the indicated concentrations. Dashed guidelines indicate the fluorescence peaks of successive divisions. B, Graphic representation of the proportion of cells in each division peak that is IgG1 (data from A). This figure is representative of four experiments.

![FIGURE 2](image2.png)  
**FIGURE 2.** Isotype switching to IgE in response to IL-4 stimulation. A, Two-dimensional contour plots of CFSE intensity vs IgE expression in lyn+/+ (left panel) and lyn−/− B cells (right panel) stimulated with CD40L and IL-4 at the indicated concentrations. Dashed guidelines indicate the fluorescence peaks of successive divisions. B, Graphic representation of the proportion of cells in each division peak that is IgE (data from A). This figure is representative of three experiments.
argued that a contaminating cell type or the lyn−/− B cells themselves may secrete factors that enhance isotype switching in these cultures. To exclude this possibility, lyn+/+ and lyn−/− B cells were cocultured with CD40L and a range of IL-4 concentrations (Fig. 3A). After 4 days of culture the proportion of each B cell type that had switched to IgG1 was determined by flow cytometry. Control and lyn−/− cells were resolved by different Ly5 alleles. The heightened responsiveness of lyn−/− B cells was again apparent as was the fact that the presence of lyn−/− cells in the culture did not enhance switching of lyn+/+ B cells. Thus, the increased sensitivity of lyn−/− B cells to IL-4 is intrinsic to the cells themselves.

Alternatively, the level of IL-4R expressed on the surface lyn+/+ and lyn−/− B cells could differ. On resting wild-type B cells there are ~100 copies of the high affinity receptor for IL-4 (29), which is below the level necessary for reliable FACS detection. Therefore, this issue was addressed using semiquantitative PCR analysis. cDNA was synthesized from the RNA extracted from purified splenic lyn+/+ and lyn−/− B cells. Some cells were cultured with CD40L and 100 U/ml IL-4 for 24 h before RNA extraction. The extracellular domain of the IL-4R α-chain and β-actin (to normalize for RNA content) were amplified using sub-saturating cycles of PCR, and the products were quantified after hybridization with internal oligonucleotide probes. The results showed that IL-4Rα expression is not enhanced in lyn−/− B cells relative to that in wild-type cells (Fig. 3B). In resting B cells the levels of IL-4Rα in lyn+/+ and lyn−/− cells were similar, being 0.4 and 0.3 the level of β-actin, respectively. Upon activation, IL-4Rα levels remained essentially unchanged relative to actin levels at 0.2 in lyn−/− and 0.3 in lyn+/+ B cells. These results indicate that differences in IL-4R levels are unlikely to contribute to the enhanced responsiveness of lyn−/− B cells to IL-4 stimulation.

IL-4 is known to enhance mitogen-induced B cell proliferation (3). To determine whether this facet of the IL-4 response was also affected by Lyn, we examined the proliferation of lyn−/− and control B cells cultured with a constant amount of CD40L and varying doses of IL-4 (Fig. 4). This measurement involved calculating the fraction of the B cell population within each division peak as revealed by the CFSE profile (for example Fig. 1A). Doing this

**FIGURE 3.** The enhanced responsiveness of lyn−/− B cells to IL-4 is intrinsic. A, Coculture of lyn+/+ (■) with lyn−/− (□) B cells in the presence of CD40L and the indicated concentrations of IL-4 for 4 days. B cells were then analyzed for surface IgG1, with the two B cell types in the mixture being distinguished by expression of different Ly5 alleles. These results are representative of three experiments. B, Quantification of IL-4Rα expression levels in lyn−/− and lyn+/+ B cells, which were unstimulated or stimulated with CD40L and 100U/ml IL-4 for 24 h. CTLL-2 cells were used as a positive control for IL-4Rα expression. Quantification was performed after hybridization with internal oligonucleotide probes as outlined in Materials and Methods.

**FIGURE 4.** B cell proliferation in response to CD40L and IL-4. Purified B cells, stimulated for 3–4 days with CD40L in the presence of the indicated concentration of IL-4, were partitioned according to cell division number using CFSE intensity. The fraction of the population within a particular division peak was calculated using the appropriate software and plotted against division number. These results are representative of four experiments.
revealed no significant difference in the proliferation of lyn−/− B cells in response to CD40L plus IL-4 compared with controls. In the absence of IL-4, lyn−/− and control B cells proliferated to an equal extent in response to CD40L. Addition of IL-4 enhanced the proliferation of both B cell types to an equal extent. This enhancement was apparent as a shift in the peak of the proliferation profile to a higher division number. That the absence of Lyn affects isotype switching and not proliferation suggests that these aspects of IL-4 signaling may be controlled by distinct mechanisms (30).

We investigated whether the heightened responsiveness of lyn−/− B cells to IL-4 had physiological significance. IgG1 AFC were detectable in lyn−/− mice at 2 wk of age and had reached adult numbers by 4 wk (Fig. 5A). In contrast, control mice had no detectable IgG1 AFC until 8 wk of age. As opposed to the lifelong elevation of IgM AFC in lyn−/− mice (data not shown), the elevated frequency of IgG1 AFC was not maintained but remained relatively normal throughout adulthood (Fig. 5A). The transient nature of the increased frequency of IgG1 AFC in lyn−/− mice may be due to either the 10-fold increase in the serum IgM concentration in adult lyn−/− mice affecting their ability to mount T-dependent responses (21) or to isotype switching to IgG1 not necessarily being an end point of differentiation, as such cells can continue to isotype switch to IgE (see below).

The spleens of adult lyn−/− mice were also examined for the proportion of surface IgG1 B cells that were not AFC. This was achieved by selecting for CD45R− cells that had undergone isotype switching, that is B cells that were IgM−IgD− and assessing the levels of IgG1 expressed. AFC have been shown to express low

FIGURE 5. Evidence for enhanced isotype switching in vivo. A, Development of IgG1 Ab-forming cells during ontogeny. IgG1 AFC were enumerated by ELISPOT for lyn+/+ (□) and lyn−/− (□□) mice from birth to 30 wk of age. B, Flow cytometric analysis of IgG1+ cells in the spleens of lyn+/+ and lyn−/− mice expressing a bcl-2 transgene. The upper panel shows total spleen staining for IgM plus IgD vs CD45R. Isotype-switched B cells, defined as IgM−IgD−CD45R−, were gated and analyzed for expression of IgG1 as shown in the lower panel. The percentage shown is IgG1-expressing B cells as a percentage of all isotype-switched B cells. C, Serum IgE levels in 8-wk-old and 10-mo-old lyn+/+ (•) and lyn−/− (□□) mice. Limit of ELISA detection indicated.
levels of CD45R and were thus excluded from this analysis (31). Although lyn−/− mice contained more IgG1+ cells than controls, this difference was not significant (data not shown). The lyn−/− B cells, however, have an intrinsically reduced life span compared with controls (32), which may prevent the accumulation of iso-type-switched B cells. We reasoned that extending the B cell life span by introducing a bcl-2 transgene expressed in the B cell lineage might allow isotype-switched B cells to accumulate in the lyn−/− mice (25). Splenks of bcl-2 transgenic lyn−/− and lyn+/+ mice were therefore examined for the proportion of IgM IgG B cells expressing surface IgG1 (Fig. 5B). This revealed a significantly higher fraction of such cells in the bcl-2 transgenic lyn−/− mice compared with bcl-2 control mice. Quantification of these data showed the difference to be significant both as a fraction of spleen and as a fraction of B cells (Table I). No surface IgG− cells were observed in either strain; however, serum analysis revealed ten-fold higher IgG1 titers in nontransgenic spleens of lyn−/− mice at both 8 wk and 10 mo of age (Fig. 5C). Thus lyn−/− mice display attributes consistent with the behavior revealed by our in vitro studies (Figs. 1 and 2).

Collectively, these results show that B cells lacking the tyrosine kinase Lyn have heightened sensitivity to IL-4 signaling. This hyper-responsiveness is B cell intrinsic and apparent in both in vitro cultures and lyn−/− mice themselves. Lyn initiates negative signaling pathways by phosphorylating ITIM (immunoreceptor tyrosine-based inhibitory motif)-containing proteins (17). ITIMs are conserved sequences that, when phosphorylated, serve as binding sites for phosphatases that become activated and suppress positive signal transduction (37), supporting the argument that Lyn and SHP-1 mechanism by which Lyn initiates negative signaling pathways at other cell surface receptors, this mode of action appears to us the less likely of the two discussed.

The abnormally high levels of IgE in the serum of lyn−/− mice indicates that setting the signaling thresholds for cytokines such as IL-4 could play a role in predisposing individuals to allergic responses. Predisposition to allergies have been extensively studied with a focus on genetic influences and the balance between Th1 and Th2 responses (5). Particular attention has been given to the levels of IL-4 and IFN-γ production during immune responses, with the logic that aberrant levels of IL-4 may lead to excessive IgE production (5) (42). Although this reasoning is valid, recent studies into the internal processing of cytokine signals provide new avenues for research into atopy (43). Although the underlying mechanism by which Lyn acts remains to be fully defined, this report is the first describing a nonreceptor protein being responsible for setting the threshold of IL-4 responsiveness and provides a means by which this issue can be pursued.

## References

7. Wang, H. Y., W. E. Paul, and A. D. Keegan. 1996. IL-4 function can be transferred to the IL-2 receptor by tyrosine containing sequences found in the IL-4 receptor α chain. Immunity 4:113.

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**Table I.** Increased frequency of surface IgG1+ B cells in bcl-2/lyn−/− mice

<table>
<thead>
<tr>
<th></th>
<th>bcl-2/lyn+/+ (n = 4)</th>
<th>bcl-2/lyn−/− (n = 3)</th>
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<tr>
<td>Splenic cellularity (×10⁷)</td>
<td>15.2 ± 5.8</td>
<td>9.1 ± 1.7</td>
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<td>B cells/spleen (%)</td>
<td>60.6 ± 8.7</td>
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<td>IgG1+ cells (%) of spleen</td>
<td>0.38 ± 0.16</td>
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<tr>
<td>IgG1+ cells (%) of B cells</td>
<td>0.64 ± 0.29</td>
<td>2.94 ± 0.86*</td>
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* Data shown are arithmetic means with standard deviations pooled from two experiments.

p < 0.005 as calculated using the Student’s t test.
LYN IS A NEGATIVE REGULATOR OF IL-4 SIGNALING


