The Transcription Factor Early Growth Response 1 (Egr-1) Advances Differentiation of Pre-B and Immature B Cells

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Summary

In mature B lymphocytes, the zinc finger transcription factor early growth response 1 (Egr-1) is one of the many immediate-early genes induced upon B cell antigen receptor engagement. However, its role during earlier stages of lymphopoiesis has remained unclear. By examining bone marrow B cell subsets, we found Egr-1 transcripts in pro/pre-B and immature B lymphocytes, and Egr-1 protein in pro/pre-B–I cells cultured on stroma cells in the presence of interleukin (IL)-7. In recombinase-activating gene (RAG)-2–deficient mice overexpressing an Egr-1 transgene in the B lymphocyte lineage, pro/pre-B–I cells could differentiate past a developmental block at the B220low BP-12 stage to the stage of B220low BP-11 pre-B–I cells, but not further to the B220low BP-11 CD25+ stage of pre-B–II cells. Therefore, during early B lymphopoiesis progression from the B220low BP-11 IL-2R+ pro/pre-B–I stage to the B220low BP-11 IL-2R+ pre-B–II stage seems to occur in at least two distinct steps, and the first step to the stage of B220low BP-11 pre-B–I cells can be promoted by the overexpression of Egr-1 alone. Wild-type mice expressing an Egr-1 transgene had increased proportions of mature immunoglobulin (Ig)M+B220high and decreased proportions of immature IgM+B220low bone marrow B cells. Since transgenic and control precursor B cells show comparable proliferation patterns, overexpression of Egr-1 seems also to promote entry into the mature B cell stage. Analysis of changes in the expression pattern of potential Egr-1 target genes revealed that Egr-1 enhances the expression of the aminopeptidase BP-1/6C3 in pre-B and immature B cells and upregulates expression of the orphan nuclear receptor nur77 in IgM+B cells.

Key words: Egr-1 • transcription factor • B cell development • BP-1 • nur77

Antigen binding to surface (s)Igs in B cells initiates a signal cascade which in the context of secondary signals leads to proliferation and differentiation of mature resting B lymphocytes into plasma or memory cells. Changes in the activity and expression of transcription factors translate activating signals into the modulated expression pattern of downstream genes. One of these transcription factors is called early growth response 1 (Egr-1;1 also known as Krox-24, NGFl-A, Tis-8, zif268, pAT225, or Z-225 [1–3]). Egr-1 is induced very rapidly in many different cell types and tissues, including fibroblasts (1), monocytes (4), lymphocytes (5, 6), kidney (7), neurons (3), and brain (8), in response to a wide range of signals (1–3, 5, 9). In mature B lymphocytes, transient Egr-1 expression is rapidly induced upon stimulation by B cell antigen receptor (BCR) cross-linking (5, 10), whereas signals resulting from Fc receptor cross-linking inhibit induction (11, 12). Thus, the broad spectrum of Egr-1 expression and the diverse modes of Egr-1 induction suggest that Egr-1 functions as a transcriptional regulator that links common biochemical signaling pathways to the rapid modulation of downstream gene expression.

Mature peripheral B lymphocytes originate from bone marrow precursor cells that are ordered according to their phenotype, gene expression, Ig gene rearrangement, and...
proliferative and developmental potential into the pro-B, pre-B, and immature B lymphocyte subsets (13–17). Transcriptional regulation plays a critical role during B cell development (for a review, see reference 18) as shown by gene targeting of multiple transcription factors. Mutations in these factors that obliterate their activity were shown to arrest B lymphopoiesis at defined stages of maturation (19–29).

Little is known about the expression and function of Egr-1 during early steps of B cell differentiation. Here we report that Egr-1 expression can be detected already in pre-B cells isolated from bone marrow and in fetal liver–derived pre-B cell cultures. These results suggested that Egr-1 might also have a regulatory function in early stages of B lymphopoiesis. However, mice deficient for Egr-1 fail to show defects in lymphocyte or monocyte maturation, most probably because the missing Egr-1 activity is masked by other members of the Egr transcription factor family (30, 31). To bypass the complementing activity of Egr-2, Egr-3, or Egr-4, we studied B lymphocyte differentiation in transgenic mice overexpressing Egr-1 in B cells in normal and recombinase-activating gene (RAG)-2-deficient mice. Since the RAG-2 mutation prevents rearrangement of Ig genes (32), precursor B cells are developmentally arrested in the stage of B220<sup>lo</sup> BP-1<sup>+</sup> pro/pre-B-I cells (33, 34). Analyzing Egr-1 transgenic RAG-2-deficient mice, we found that pro/pre-B-I cells overcame the RAG-2<sup>–/–</sup> induced differentiation block at the stage of B220<sup>+</sup> BP-1<sup>+</sup> pro/pre-B-I cells and differentiated into B220<sup>+</sup> BP-1<sup>+</sup> pre-B-I cells. Comparing B lymphocyte maturation in the bone marrow of normal transgenic and control animals, we found that Egr-1 transgenic mice had increased their fraction of mature cells. Because Egr-1–enhanced progression of developing thymocytes was also found in transgenic mice overexpressing Egr-1 in T cells (35), we propose that Egr-1 activity promotes maturation of B and T lymphocytes.

**Materials and Methods**

**Pre-B Cell Cultures.** Fetal liver cells of day 15–18 embryos were removed and plated onto irradiated ST-2 feeder cells in Iscove’s medium containing IL-7 and 10% FCS. Cells were cultured as described previously (36). Cells from transgenic lines were identified by PCR. For further analyses, nonadherent cells were collected and washed twice in ice-cold PBS. Samples from wells containing only ST-2 feeder cells were treated in parallel and served as controls.

**Mice.** The detailed description of the generation of Egr-1 transgenic mice using the BALB/c embryonic stem cell line BALB/c-c1 will be described elsewhere. Egr-1 transgenic mice of the IA7 line were transferred to a special pathogen-free unit by implanting transgenic one-cell embryos into C57BL/6 foster animals kept under specific pathogen–free conditions. The IA7 line were transferred to a special pathogen-free unit by implanting transgenic one-cell embryos into C57BL/6 foster animals kept under specific pathogen–free conditions. The IA7 line were then bred further by mating with wild-type BALB/c mice. The detailed description of the generation of Egr-1 transgenic mice using the BALB/c embryonic stem cell line BALB/c-c1 will be described elsewhere. Egr-1 transgenic mice of the IA7 line were transferred to a special pathogen-free unit by implanting transgenic one-cell embryos into C57BL/6 foster animals kept under specific pathogen–free conditions. The IA7 line were then bred further by mating with wild-type BALB/c mice.

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**RNA Isolation, Northern Blot, and PCR Analysis.** RNA was extracted using the guanidium isothiocyanate method as described (37). For Northern blotting, 10 μg of total RNA was separated in a 1% agarose gel containing 7% formamide, transferred onto nylon filters, and fixed by UV cross-linking. Filters were prehybridized (50% deionized formamide, 5× SSC, 5× Denhardt’s solution, 50 mM Na<sub>2</sub>HPO<sub>4</sub>, pH 7, 10 mM Na<sub>2</sub>HPO<sub>4</sub>, 0.1% SDS, 0.1 mg/ml denatured salmon sperm DNA) for 2 h at 42°C. For detection of Egr-1–specific transcripts, [α-32P]dATP–labeled probes were prepared from a 1.6-kb EcoRI-HindIII fragment from plasmid 533 (a gift from V. Sukhatme) containing the Egr-1 cDNA by the oligonucleotide priming method (38). The probe was added to the prehybridization and filters were incubated overnight, washed with 0.2× SSC, 0.1% SDS at 42°C, and exposed to X-ray films. Egr-1 expression was analyzed by PCR using cDNA reverse transcribed from total RNA with SuperScript II (GIBCO BRL, Eggenstein, Germany) and the Egr-1–specific primers 5′-GCATTGCTCTGACCCTGCCG-3′ and 5′-CCGACGCTTTGCTGCGTAT-3′ as described by T. Miyazaki (35). PCR was performed using Taq polymerase (M BI Fermentas, Inc., Amherst, NY) using 1/25 of the cDNA reaction template at an annealing temperature of 54°C.

**Immunoblot Analysis.** Bone marrow cells from six femurs were isolated and resuspended in FACS buffer (0.1% sodium azide, 3% FCS in PBS). B220–specific biotin–labeled antibody R A3.3A1 (39) was added and incubated for 30 min on ice. Cells were washed, magnetic streptavidin–labeled beads (Dynal, Oslo, Norway) were added, and B cells were isolated. Quality of the sorting process was verified by flow cytometric analysis. The B cells were resuspended in 30 μl lysis buffer (1% NP-40, 150 mM NaCl, 10 mM Tris-HCl, pH 7.0, 0.1 mM PM SCF) and incubated on ice for 10 min. Cell debris was removed by centrifugation (10 min, 4°C, 22,000 g), and the extract was separated by SDS-PAGE (8%) and transferred onto nitrocellulose membrane (Hybond C extra, Amersham Pharmacia Biotech, Uppsala, Sweden). Egr-1 was detected using the antisemur C19 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) at 25 ng/ml followed by peroxidase–conjugated goat anti–rabbit IgG F(ab’)<sub>2</sub> (Dianova GmbH, Hamburg, Germany) at 200 ng/ml. Expression of nur77 was analyzed using a mouse IgG anti-nur77 mAb (a gift of B. O’born, University of Massachusetts, Amherst, MA) followed by peroxidase–conjugated goat anti–mouse IgG (Southern Biotechnology Associates, Birmingham, AL). IgM was detected by a goat anti–mouse IgM peroxidase–labeled serum (Southern Biotechnology Associates, Inc.). Signals were visualized using an enhanced chemiluminescence (ECL) detection system (Amersham Pharmacia Biotech).

**Flow Cytometry.** Flow cytometry was carried out as described previously (40) using the following antibodies: R53.1–biotin specific for murine IgM (41), 6C3–biotin for BP-1, 7D4–biotin for IL-2R α chain, 2B8–biotin for c-kit, S7–biotin for leukosialin, AM S9.1–biotin for IgD<sup>lo</sup>, R A3-6B2–PE for B220, IM 7–biotin for Pgp-1, 3E2–PE for intercellular adhesion molecule 1 (ICAM-1) (all from PharMingen Europe, Hamburg, Germany), and biotinylated PB493 (42) to stain immature B lymphocytes. Cells were counterstained using PE- or APC–conjugated streptavidin (PharMingen Europe, Hamburg, Germany) at 200 ng/ml. Expression of nur77 was analyzed using a mouse IgG anti-nur77 mAb (a gift of B. O’born, University of Massachusetts, Amherst, MA) followed by peroxidase–conjugated goat anti–mouse IgG (Southern Biotechnology Associates, Inc.). Signals were visualized using an enhanced chemiluminescence (ECL) detection system (Amersham Pharmacia Biotech).

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sion in bone marrow B cell subsets, 3.4 × 10^7 pre-B and 7 × 10^6 immature B cells were isolated from both femurs of a 5-wk-old BALB/c mouse by cell sorting at 4°C according to their IgM^+ PB493 staining pattern using a FACStar® cell sorter and LYSIS II® software (Becton Dickinson).

Bromodeoxyuridine Treatment and Staining. Bone marrow cells were labeled with bromodeoxyuridine (BrdU; Sigma, Deisenhofen, Germany) starting with a single injection of 1 mg/ml i.p. BrdU and feeding mice continuously with drinking water containing 1 mg/ml BrdU for 48 h as described (43). During the labeling period, the drinking water was protected from light. Simultaneous detection of surface staining and BrdU labeling was done as described (44). After surface marker staining, cells were resuspended in 500 μl 0.15 M NaCl, 1.2 ml ice-cold 95% ethanol was added, and the cells were incubated 30 min on ice. Cells were washed and resuspended in 1 ml 0.15 M NaCl, pH 5. Cells were washed, 10 μl anti-BrdU antibody (Becton Dickinson) was added, and the cells were then incubated for 30 min and washed. Electrophoretic Mobility Shift Assays. Gel shift was carried out using recombinant Egr-1 as described (45) with double-stranded radiolabeled oligonucleotides from the nur77 and BP-1 promoter regions carrying putative Egr-1 binding sites (bold): 5'-TTC-CAAACTCCCTCCAAGCCGC-3' for BP-1 (position -753 to -729), 5'-GTACTGCGCCCGGCGGCTCTCTCTGCGGCGGCGTCTCTCTC-3' for nur77 (position -66 to -50), and 5'-GGATCTCAGCCGGGCGAAGCCGGGGCG-3' for Egr-1.

Results

Egr-1 Expression in Pre-B and Immature B Cell Precursors. BCR cross-linking has previously been reported to induce Egr-1 expression in mature B cells, but not in immature B lymphocytes or in immature B cell lines (5, 46, 47). We addressed the question of whether unstimulated pre-B and immature B cells express Egr-1 by analyzing sorted B cell subsets from murine bone marrow. Transcription of the Egr-1 gene was found by PCR in both sIgM^- (pre-B) and sIgM^+ PB493^- immature B cells (Fig. 1 A). Likewise, Egr-1 protein was detected by immunoblotting in sIgM^- pre-B cells isolated from fetal liver and expanded in culture on ST-2 stroma cells in the presence of IL-7 (Fig. 1 B). Both results show transcription of the Egr-1 gene and translation of Egr-1 mRNA into detectable amounts of protein as early as the pre-B cell stage before BCR surface expression.

Egr-1 Expression in Transgenic and Normal Mice. These results suggested that Egr-1 function might also be important during early stages of B lymphopoiesis. To test this hypothesis, we generated transgenic mice expressing Egr-1 specifically in B lymphocytes using an Ig heavy chain promoter/enhancer construct. Four different founder mice showing Egr-1 germline transmission were obtained. By breeding to BALB/c mice, we established the Egr-1 transgenic lines IA7, IB10, IC4, and ID4 (to be published elsewhere). At first, we compared Egr-1 expression between transgenic and BALB/c control mice by Northern and immunoblotting (Fig. 2). Spleen cells of the line IA7 expressed 10-fold more Egr-1 mRNA than the control littersmates, whereas the other lines showed Egr-1 expression levels of about two- to threefold above unstimulated spleen cells (Fig. 2 A). Since transgenic IC4 mice expressed only low levels of Egr-1 they were abandoned. Carrying out most of the experiments with mice from lines IA7, IB10, and ID4, we found only small variations between these transgenic lines. Testing Egr-1 protein expression, we found high levels in purified B220^+ bone marrow B cells as well as in cultivated pre-B cells isolated from fetal liver (Fig. 2, B and C, respectively).

These results show that transgenic Egr-1 is expressed during similar stages of B cell maturation, but at far higher levels than endogenous Egr-1.

Egr-1 Expression Promotes At Least Two Different Stages of B Cell Development. To examine whether enhanced Egr-1 expression has an effect on early stages of B cell development, we backcrossed the IA7 transgenic mice to a RAG-2-deficient background. The RAG-2 mutation prevents rearrangement of the Ig genes (32) and therefore blocks B cell maturation at the pro/pre-B-I cell stage (16, 34). These cells carry the surface markers c-kit and CD43, <5–15% express BP-1 and <1% express the I-2R α chain (data not shown). Phenotypically, these pro/pre-B-I cells correspond to fraction B as classified by Hardy et al. (13). FACScan® analysis of control and Egr-1 transgenic RAG-2-deficient mice revealed an unchanged expression pattern for c-kit and CD43, but a three- to fourfold increase in the fraction of BP-1^+ cells, compared with control littersmates (Fig. 3). Phenotypically, these BP-1^- c-kit^- CD43^- pre-B lymphocytes are defined as fraction C cells (13), and progression into this stage normally requires RAG-2 expression and Ig heavy chain gene rearrangement (16, 34), suggesting that Egr-1 might support the maturation of fraction B pre-B cells even in the absence of RAG-2 activity. The reduced cell size of transgenic BP-1^- B220^+ lymphocytes as reflected by changes in forward/side scatter (Fig. 3 A, c
Figure 3. Egr-1 induces development of BP-1<sup>+</sup> B220<sup>low</sup> pre-B cells. Bone marrow cells of IA7 transgenic mice with a RAG-2 background and control littermates were stained with BP-1<sup>+</sup>- and B220-specific antibodies and analyzed by flow cytometry. Dead cells were excluded by gating for propidium iodide-negative cells, and 5 x 10<sup>4</sup> cells were acquired according to their forward/side scatter (FSC, SSC) profile. As shown for one individual example in A, IA7 mice (b) have about three times more BP-1<sup>+</sup> cells than control littermates (a). Changes in the forward/side scatter pattern document that most of the B220<sup>+</sup> BP-1<sup>+</sup> cells (74% small cells, d) are smaller than the majority of BP-1<sup>-</sup> cells (37% small and 54% large cells, c). B compiles the BP-1 staining pattern for six RAG-2-deficient IA7 mice and six control littermates.

Figure 2. Expression of transgenic Egr-1. (A) Comparison of Egr-1 mRNA levels in BALB/c and Egr-1 transgenic spleen cells. RNA was extracted from splenocytes, and Northern blot analysis was performed using a probe specific for endogenous and recombinant Egr-1 mRNA. Because the endogenous and transgenic Egr-1 mRNA species migrate with different electrophoretic mobilities, they are easily identified on Northern blots (data not shown). To standardize for the amounts of mRNA, filters were rehybridized with a GAPDH-specific probe. The relative intensity of the Egr-1 expression in BALB/c and in four transgenic lines IA7, IB10, IC4, and ID4 was determined using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). (B) Expression of pre-B cell cultures and immunoblots were performed as described for Fig. 1 B. Transgenic pre-B cells (IA7, lane 3) express higher levels of Egr-1 than an equal amount of wild-type BALB/c pre-B cells (lane 2). Egr-1 protein is undetectable in whole cell lysates of corresponding numbers of ST-2 feeder cells (lane 1).
sites. Finding a (5'-GAGGGGGGAA) sequence ~1.6 kb upstream of the mRNA start (50) resembling an Egr-1 binding site (5'-GCCGCGCGGC), we analyzed by an electrophoretic mobility shift assay (EMSA) if recombinant Egr-1 binds to an oligonucleotide containing the putative Egr-1 recognition site from the BP-1 promoter. As shown in Fig. 6C, labeled oligonucleotides containing a cognate Egr-1 binding site (lane 1) or the binding site from the BP-1 promoter (lane 15) produced a shifted DNA–protein complex with identical electrophoretic mobility. Their intensities were reduced only by adding an excess of unlabeled oligonucleotides with an Egr-1 binding site but not by competing with an Sp-1 binding site (lanes 2–5 and 16–19, respectively). Likewise, only the addition of Egr-1– but not of Sp-1–specific antibodies retarded the migration of the complex (lanes 6 and 7, 20 and 21). Therefore, the forced expression of Egr-1 in transgenic B cells may not only help pre-B cells to proceed from fraction A into fraction C, but may also enhance the expression of BP-1, which is normally upregulated during this transition.

The nur77 gene (also called NGFI-B [3] or N10 [51]) encoding an orphan nuclear receptor represents one of the transcription factors found to be induced by Egr-1 (52). Analyzing nur77 expression in purified B220–bone marrow cells of transgenic (ID4) and control littermates by Western blotting, we found nur77 to be expressed only by transgenic but not by control B cells (Fig. 6B). Since up-regulated nur77 expression was not found in cultivated transgenic sIgM + pre-B cells (data not shown), we conclude that nur77 is expressed only by pre-B cells and not by mature B220–B cells.

Figure 4. Higher frequency of immature bone marrow cells in Egr-1 transgenic mice. (A) Comparison of the differences between the B220low IgM + (immature) and B220high IgM + (mature) B cell subsets of control littermates and transgenic mice. Bone marrow cells of 4- to 40-wk-old BALB/c littermates (n = 23) and of IA7 transgenic mice (n = 23) were stained for IgM (R53.1) and B220 (RA3-6B2) and analyzed by flow cytometry after acquisition of 3 × 10^6 cells gated according to their forward/side scatter profile. The first diagram (a) compares the changes in the percentage of immature IgM + B220low cells between age-matched BALB/c littermates (open circles) and IA7 mice (filled circles) over a period of 36 wk. P values <0.05 corresponding to the individual time points indicate statistically significant differences between transgenic and control mice. The second diagram (b) shows the increase in the percentage of mature IgM + B220high cells in older mice. Although transgenic IA7 mice tend to have more mature bone marrow B cells, they do not show statistically significant differences seen for the immature B cells. (B) Two individual examples of a BALB/c littermate (a) and an IA7 transgenic mouse (b) at 18 wk. The numbers indicate the percentage of B220– cells in each subset. Similar analyses of bone marrow B cells from the IB10 and ID4 transgenic mice showed almost identical results.

Discussion

Egr-1 accelerates B cell maturation. Mature B cells respond to signals resulting from antigen receptor engagement by immediately inducing Egr-1 transcription (5), but the role of Egr-1 in earlier stages of B cell development has
Egr-1 advances B cell development. The staining shows elevated nur77 expression in ID4. Size markers (in kD) as indicated were run in parallel to the samples. (C) Binding of recombinant Egr-1 to sequences present in the BP-1 and nur77 promoters. Recombinant Egr-1 was incubated with radioactively labeled oligonucleotides carrying a cognate Egr-1 binding site (lanes 1–7, Egr-1), with an oligonucleotide from the nur77 promoter (lanes 8–14, nur77), or with an oligonucleotide from the BP-1 promoter (lanes 15–21, BP-1) and analyzed by EMSA as described previously (reference 45). The sequences of the respective Egr-1 binding sites are shown (top). Specific binding was proven first by competing either with an excess of an unlabeled oligonucleotide carrying a cognate Egr-1 binding site (lanes 4, 5; 11, 12; 18, 19) or by using an oligonucleotide with an Sp-1 site (lanes 2, 3; 9, 10; 16, 17), and second by inducing a "supershift" by adding the Egr-1–specific antibody C19 to the binding reaction (lanes 6, 13, and 20). Replacement of the Egr-1–specific antibody with an Sp-1–specific antibody had no effect on the migration of the DNA–Egr-1 complex (lanes 7, 14, and 21).

not be defined. The different stages and the order of B cell development are well characterized, allowing the precise typing of bone marrow B cells according to the expression of characteristic cell surface markers, the rearrangement of Ig genes, and the proliferative and differentiation potential of B cell precursors (13, 14, 16, 60, 61). By analyzing Egr-1 expression in bone marrow–derived B lymphocyte subsets and by testing Egr-1 expression in cultured, fetal liver–derived pre-B cells, we have shown that Egr-1 is also expressed in pre-B cells lacking sIgM as well as in immature sIgM + B cells in the absence of sIgM–induced signals. These observations suggest that Egr-1 might also have a regulatory function in pre-B cell development. By studying transgenic mice overexpressing Egr-1 from the pre-B stage on, we have found higher proportions of mature B cells and fewer immature B cells in transgenic animals than in control littermates. To identify if early stages of B lymphopoiesis are sensitive to Egr-1 activity, we arrested B cell development at the stage of pro/pre-B–I cells by backcrossing the Egr-1 transgenic line IA7 to mice deficient in RAG-2. Since the null mutation in the RAG-2 gene prevents rearrangement of Ig genes (32), B cell precursors do not receive stimulating signals required for developmental progression beyond the stage of B220low CD43+BP-1+ pro/pre-B cells (16, 62), also defined as fraction B (13). Comparing the phenotype of bone marrow pro/pre-B cells from transgenic and control mice, we found a three- to fourfold increased population of BP-1+ pre-B cells in Egr-1 transgenic mice. Since the transcription activation function of Egr-1 seems to enhance BP-1 expression in more mature B cell subsets, the increase in BP-1+ pre-B cells could also reflect the induction of BP-1 expression only and not Egr-1–induced differentiation. However, this seems to be less likely because transgenic BP-1+ cells were found to be smaller than BP-1+ cells, consistent with further maturation. Therefore, these results suggest that forced expression of Egr-1 in BP-1+ pro/pre-B cells induces progression into the stage of BP-1+ pre-B cells (fraction C). Since these cells failed to upregulate the IL-2Rα chain and heat-stable antigen, two markers characteristic for pre-B–II cells (fraction C′ and D [13, 16]), overexpression of Egr-1 in pro/pre-B–I cells seems to be sufficient to induce differentiation to fraction C, but not to more mature stages of B lymphopoiesis.

Progression of pro/pre-B cells developmentally arrested by a mutation in the RAG-2 gene into more mature pre-B cell stages is also induced by in vivo cross-linking of the Igα/Igβ heterodimer using Ig-β–specific mAbs (63). Under those conditions, anti-Ig-β–treated pro/pre-B–I cells become smaller in size and acquire IL-2Rα expression in addition to BP-1. Since they also downregulate c-kit (CD117) and CD43, they are considered as small pre-B–II cells. In the same report, it was shown that Ig-β cross-linking stimulates tyrosine phosphorylation of several substrate
proteins, including Ig-α, Syk, and Vav, and the activation of mitogen-activated protein kinase extracellular signal-regulated kinase (ERK) 1. Based on these results, Nagata et al. (63) proposed that the signal cascade initiated by Ig-β activation evokes differentiation signals similar to those delivered by the pre-BCR in normal B cell development. For mature B cells it is known that BCR engagement upregulates Egr-1 transcription through a signal cascade including p21/ras and mitogen-activated protein kinase (ERK [10, 64]), and for other cell types it has been shown that ERK activation induces Egr-1 transcription (65). Since RAG-2–deficient pro/pre-B–1 cells overexpressing Egr-1 do not reach the same developmental stage as anti-Ig-β-stimulated cells, it seems likely that Egr-1 activity substitutes only part of the differentiation signal originating from the pre-BCR.

Analyzing later stages of B cell development in RAG-2+/+ Egr-1 transgenic mice, we observed lower proportions of immature and increased proportions of mature bone marrow B cells compared with their wild-type littermates, whereas there was no increased proliferation of transgenic pre-B or immature B cells detectable. These findings are consistent with the current model of the development from immature to mature B cells (66, 67). Immature B cells leave the bone marrow and enter the spleen where about half of them reach the mature stage (42). Mature bone marrow B cells are thought to be part of the recirculating pool. This would suggest that Egr-1 influences this migration at one or several steps.

Egr-1 expression was also found in CD4−CD8− double negative thymocytes by Miyazaki (35). Overexpression of transgenic Egr-1 in a RAG-2–deficient background allowed thymocytes to bypass the RAG-2–dependent block at the IL-2R−Pgp-1− double negative stage and develop into immature CD8 single-positive cells, but not further to the CD4+CD8+ double-positive cell stage. In cortical CD4+CD8+ thymocytes, Egr-1 expression was reported by Shao et al. (68) to be dependent on TCR engagement, suggesting that high level expression of Egr-1 in the thymus might be a consequence of thymocyte selection. The high coincidence of Egr-1 expression in analogous B and T cell precursor subsets and the increased differentiation of pro/pre-B–1 cells and thymocytes in Egr-1 transgenic mice suggest that Egr-1 activity regulates similar functions in both types of lymphocytes.

Conclusions. Here we provide evidence that Egr-1 supports at least two distinct steps of B cell maturation, the progression into the pre-B and into the mature B cell stage. Since Egr-1 activity is also sufficient to promote the development of double negative thymocytes into immature single-positive CD8low cells (35), as well as macrophage in vitro differentiation (73, 74), this transcription factor seems to play an important role in the differentiation of three major hematopoietic cell types.
References


