Serum Response Factor Contributes Selectively to Lymphocyte Development*

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Serum response factor (SRF), is a crucial transcription factor for murine embryonic development and for the function of muscle cells and neurons. Gene expression data show that SRF and its transcriptional cofactors are also expressed in lymphocyte precursors and mature lymphocytes. However, the role of SRF in lymphocyte development has not been addressed in vivo so far, attributed in part to early embryonic lethality of conventional Srf-null mice. To determine the in vivo role of SRF in developing lymphocytes, we specifically inactivated the murine Srf gene during T or B cell development using lymphocyte-specific Cre transgenic mouse lines. T cell-specific Srf deletion led to a severe block in thymocyte development at the transition from CD4/CD8 double to single positive stage. The few residual T cells detectable in the periphery retained at least one functional Srf allele, thereby demonstrating the importance of SRF in T cell development. In contrast, deletion of Srf in developing B cells did not interfere with the growth and survival of B cells in general, yet led to a complete loss of marginal zone B cells and a marked reduction of the CD5⁺ B cell subset. Our study also revealed a contribution of SRF to the expression of the surface molecules IgM, CD19, and the chemokine receptor 4 in B lymphocytes. We conclude that SRF fulfills essential and distinct functions in the differentiation of different types of lymphocytes.

Serum response factor (SRF) is a widely expressed transcription factor belonging to an ancient family of DNA-binding proteins. Its activity is regulated by the interaction with transcriptional cofactors, some of which are expressed in a cell type-selective fashion (2). SRF interacts directly with at least two classes of signal-regulated cofactors, the ternary complex factor (TCF) subfamily of Ets domain proteins (SAP-1, Elk-1, and Net) which respond to mitogen-activated protein kinase (MAPK) signaling (3) and members of the myocardin-related transcription factor (MRTF) family (4–6), which may be regulated through Rho GTPase/actin signaling. SRF activity can be triggered by a multitude of means such as serum, ionizing radiation, growth factors, and intracellular calcium-regulating agents. A steadily increasing set of SRF target genes is being identified, which includes immediate early genes (IEGs), cytoskeletal protein-encoding genes, and muscle differentiation genes (7, 8). Regarding cellular function, recent in vivo and in vitro studies revealed essential contributions of SRF to murine embryogenesis (9), neuronal development (10–12), heart development (13–15), skeletal muscle function (16, 17), programmed cell death (18, 19), and processes of cell morphogenesis, adhesion, and migration (20).

Cell culture analyses point to an additional requisite role for SRF in lymphocyte function and development. SRF is widely expressed in hematopoietic cell lines (21, 22), activated in response to various cytokines (23), and responds to signaling via the receptors of T cells (24–26) and B cells (27, 28). Furthermore, SRF is involved in the regulation of lymphocyte-specific genes (e.g. Il2, Il2Rα, Ifnγ) (24–26). MAP kinase and Rho GTPase signaling, which have been shown to trigger SRF responses (2), are vitally important for the selection, proliferation, and maturation of thymocytes (24, 29–32) and B cells (27, 28, 33).

Stimulation of lymphocyte antigen receptors (B cell receptor (BCR) and T cell receptor (TCR)) rapidly induces expression of c-fos and early growth response factor 1 (egr-1) (28, 33, 34), which are immediate early target genes of SRF. Several studies support the importance of these genes for lymphocyte development: Overexpression of c-fos in mice augments the differentiation and accumulation of peritoneal B1b cells (35), marginal zone B (MZB) cells (36), and terminally differentiated antigen-specific B cells (37). Activator protein 1 is composed of members of the Fos and Jun family of DNA-binding proteins. Induction of activator protein-1 is required for activation of the germline κ promoter. This represents an essential step preceding immunoglobulin (Ig) isotype switching to IgE (38) and plays a central role in the regulation of promoter activity for interleukin...
kin-2 (IL-2) and interferon-γ in T cells (25). The immediate early gene ets-1 has been shown to play a role in limiting the number of T cell precursors and in the efficient differentiation and survival of thymocytes during the process of positive selection (39).

SRF has the potential to regulate cell growth, survival or apoptosis by altering the expression of specific genes involved in these processes. Experiments in a human B cell line demonstrated that for apoptosis to proceed, the transcriptional events promoting cell survival and proliferation in which SRF is involved must first be inactivated by a caspase-mediated cleavage of the SRF protein (18). One mechanism by which SRF inhibits apoptosis is the transcriptional regulation of members of the B cell leukemia/lymphoma (Bcl) family of anti-apoptotic genes (Bcl-2, Bcl-xL, and Myeloid cell leukemia sequence 1 (Mcl-1)). Neither Bcl-xL nor Bcl-2 is absolutely required for T cell development to maturity (40–42). Conditional Mcl-1 mutants, however, display a profound reduction in B and T lymphocytes (43).

TCFs are thought to function primarily through serum response elements via formation of ternary complexes with SRF. Recently, members of the TCF family of SRF cofactors have been characterized by knock-out studies. Elk-1 deficient mice display normal immune responses and mildly impaired neuronal gene inactivation (44) and Net mutants show defects in cell migration (45), vasculature development (46), and impaired angiogenesis during wound healing (47). The strongest phenotype with regard to immune functions is observed in SAP-1-null mice where thymocyte development is severely impaired and a decrease in the amount of CD4+ and CD8+ single positive (SP) cells is seen resulting from defective thymocyte positive selection (48). The relatively mild phenotypes observed upon inactivation of single TCFs suggest functional redundancy between the different TCFs. Gene targeting in mice has been particularly helpful in deciphering the genetic networks underlying lymphocyte development and function.

Classical disruption of both Srf alleles in mice leads to early embryonic lethality associated with a gastrulation defect (9), thereby precluding the analysis of SRF function in subsequent developmental processes. To investigate potential contributions of SRF to lymphocyte differentiation and function, we conditionally ablated the Srf gene in developing lymphocytes of the mouse. Potential functional redundancies of TCF proteins are thereby also addressed since SRF mutagenesis inactivates all members of the B cell leukemia/lymphoma (Bcl) family of anti-apoptotic genes (Bcl-2, Bcl-xL, and Myeloid cell leukemia sequence 1 (Mcl-1)). Neither Bcl-xL nor Bcl-2 is absolutely required for T cell development to maturity (40–42). Conditional Mcl-1 mutants, however, display a profound reduction in B and T lymphocytes (43).

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EXPERIMENTAL PROCEDURES

Lymphocyte Specific Deletion of Srf in Mice—Animals were bred at the Helmholtz Centre for Infection Research under specific pathogen-free conditions and all animal experiments were performed in accordance with institutional guidelines. Srf was deleted specifically in murine lymphocytes by using mice carrying the conditional Srf allele Srffllox/neo (abbreviated fl) which is converted into the Srflox allele (abbreviated lox) by Cre-mediated recombination (49). Conditional Srffl mice (49) were bred to CD4-Cre (50) or CD19-Cre (51) mice on a mixed 129Sv/Ei/C57Bl/6 background. Detection of the Srflox allele was performed by polymerase chain reaction (PCR) using two primer combinations (SRF-E/R and SRF-L/R) as previously described (49). The Cre transgene was detected by PCR using primer 1 (5′-ACGACAAAGTGACGACATG-3′) and 2 (5′-CTCAGACAGTTAGTTACC-3′). Srf deletion was assessed by Southern blot analysis performed on BglII-digested genomic DNA isolated from FACS-sorted lymphocytes. As described previously (49), hybridization with an external 3′ probe allowed discrimination between wt (4.6 kb), fl (3.8 kb), and lox alleles (1.4 kb).

Histology—Formalin-fixed organs were embedded in paraffin and sectioned at 4 μm thickness. Immunohistochemistry for T lymphocytes was performed with a rat-anti-CD3 antibody (CD3–12, Serotec Ltd.). A biotinylated rabbit-anti-rat antibody was used for detection of the bound primary antibody. B lymphocytes were detected with a biotin-conjugated rat-anti-mouse-CD45R/B220 monoclonal antibody (RA3–6B2, BD Biosciences). The avidin-biotin complex (ABC) method with diaminobenzidine as chromogen was used for detection of the biotinylated antibody.

For cryosections, spleens were embedded in OCT freezing medium on dry ice, and 10-μm sections were prepared. Air-dried sections were fixed and subsequently stained with a mixture of fluorescent dye-coupled antibodies containing rat-anti-MOMA-FITC (MCA947F, Serotec, Oxford, UK), rat-anti-mouse-IgM-Alexa 594 (clone R33–24), and mouse-anti-mouse-Ig-D-Alexa 647 (clone 1.3) in Tris-buffered saline-Tween 20 (TBST) with 2% rat serum. Pictures were processed with Photoshop software (version 10.0 SE, Ulead Systems).

Flow Cytometry—Single cell suspensions were prepared from thymus, spleen, lymph nodes, peritoneal cavity, and bone marrow. Peripheral blood was taken from the tail vein and heparinized using 375 I.E. heparin-sodium. Erythrocytes were depleted by lysis. After washing, cells were stained with various combinations of antibodies. To exclude dead cells, propidium iodide was used. The cell suspensions were measured on a FACS-Calibur flow cytometer (BD, San Jose, CA). Data were analyzed by FlowJo software (version 6.3.2 and 6.4.2). For cell sorting, cells were analyzed and collected on a MoFlo cell sorter (DakoCytomation). The following antibody conjugates were used: anti-CD4–PE-Cy5, anti-CD8–PE-Cy5, anti-Gr-1–PE, anti-Gr-1–FITC (eBioscience, San Diego, CA), anti-CD19–APC, anti-CD49–FITC, anti-CXCR4–Bio, anti-CD21–35–FITC, anti-CD23–PE, anti-CD8–FITC, anti-CD5–Cy (BD Biosciences Pharmingen), anti-IgM–PE, anti-IgD–FITC, and anti-F4/80–PE (Serotec, Oxford, UK). The biotinylated anti-CXCR-4 antibody was detected by streptavidin-PE (BD Biosciences Pharmingen).

Electrophoretic Mobility Shift Assay (EMSA)—EMSA studies were performed as described previously (52). A [α32P]ATP-labeled DNA fragment containing the c-fos serum response element (SRE) was used as a DNA-binding probe either with 4 μg
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**RESULTS**

**T Cell Lineage-specific Deletion of Srf**—To delete Srf specifically in lymphocytes we used a conditional Srffl mouse strain where Cre-mediated recombination results in formation of the Srflx allele deleting the complete coding region of exon 1 (49).

To the best of our knowledge, there is not a protein product derived from this allele. However, the presence of such a truncated protein cannot be ruled out with 100% certainty (13). If such a hypothetical product were derived from the Srffl locus, it would be predicted to have lost all the important functions of nuclear localization, dimerization, and DNA binding. To restrict deletion of the Srffl allele to developing T cells, the lymphocyte-specific CD4-Cre transgene (50) was introduced by breeding. The resulting Cre expression is directed by the murine CD4 enhancer/promoter/silencer, which leads to efficient Cre-mediated deletion in CD4+CD8+ and CD4−CD8− T cells, and, to a lesser extent, in CD4+CD8− T lymphocytes (55). The resulting mutant CD4-CreSrfflmice when compared with littermate control mice (i.e. Srffl, Srffl/wt, and CD4-Cre-Srffl/wt) (data not shown). The reduction of peripheral T cells suggested an impaired T cell development in the thymus. The overall number of thymocytes was similar in wild-type and SRF-deficient mice and no major differences in DN and DP thymocyte populations were detected by FACS analysis. We observed, however, a dramatic reduction of CD4+ (−80%) and CD8+ (−50%) T cells among the thymocytes from SRF-deficient CD4-CreSrffl mice (Fig. 1B).

Peripheral T Cells Lacking SRF Are Non-Viable—The remaining T cells may have survived without functional SRF or may represent a minority population that failed to delete the Srf gene. To address this question we sorted T and B cells from thymus and spleen and performed Southern blot analysis to distinguish the active Srffl allele from the inactive Srffl allele (Fig. 2A). Consistent with the absence of CD4+ thymocytes in B cells, only the Srffl, but not the deleted Srffl allele could be detected in B cells from spleens of CD4-Cre-Srffl mice (Fig. 2A, right panel). In contrast, the Srffl allele was completely recombined to Srfsl in CD4+CD8+ DP cells from thymus of CD4-CreSrffl mice (Fig. 2A, left panel). Thus, CD4-Cre-mediated excision of Srffl resulted in selective perturbations of T cell populations. The Srfsl allele was also detectable in peripheral T cells isolated from the spleens of mutant animals (Fig. 2A, center). However, in these splenic T cells we detected an additional band, which was specific for the intact Srffl allele. We then decided to investigate SRF protein content of lymphocyte cells directly by EMSA. Protein extracts prepared from CD4+CD8− DN cells (in which Cre is not yet expressed) from thymi of mutant mice contain SRF as shown by the protein-DNA complex in Fig. 2B (left panel). In contrast, no SRF-DNA complex was visible when we used CD4+CD8+ DP cell extracts from CD4-CreSrffl mice. The presence of SRF protein in these shifted complexes was verified by supershift experiments with SRF antiserum. To prove the general capability of our protein extracts for specific DNA binding, we performed EMSA control experiments employing a tandem DNA binding site for the
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NFAT transcription factor (data not shown). Furthermore, when lymphocyte extracts from spleen were used for gel shift assays we detected SRF protein not only in B cells of CD4-Cre Srffl/fl mice, but also in the remaining peripheral T cells (Fig. 2B, right panel). The latter result is in agreement with the Southern blot analysis, indicating the presence of the non-recombined Srf allele in some splenic T cells. Taken together, our data suggest that peripheral T cells lacking both copies of Srf are non-viable and that the residual T cells found in CD4-Cre Srffl/fl mice are derived from cells that have escaped Cre-mediated deletion.

B Lineage-specific Srf Deletion—The CD19-Cre mouse strain used here for B cell-specific recombination of the Srf allele has been generated by a knockin of the Cre recombinase into the CD19 locus. CD19-Cre hemizygous mice are phenotypically normal and can be used for B lineage-specific deletion of a floxed target gene (56). To disrupt Srf specifically in B cells we crossed CD19-Cre mice with Srffl/wt animals. Mutant CD19-Cre Srffl/fl mice were born with Mendelian distribution and appeared phenotypically normal. Southern blot analysis showed a complete B cell-specific deletion of the Srf allele in FACS-sorted splenocytes of CD19-Cre Srffl/fl mice (Fig. 3A). Presence of SRF in the protein-DNA complexes was monitored by EMSA, including supershift assays with SRF antiserum (Fig. 3B). Again, NFAT DNA-binding was used in control EMSAs to check for the integrity of cell extracts (data not shown). The remaining SRF protein in splenic B cells from CD19-Cre Srffl/fl mice is in contrast to the complete recombination of Srf seen in the Southern blot analysis and can probably be attributed to the long half-life of the SRF protein (15, 57). This has also been noticed in embryonic myocytes (15) and in fibroblasts where a half-life of at least 12 h was estimated for SRF (57).

B Cell-specific Srf Deletion Leads to a Decrease in B Cell Numbers, CD19, and IgM Expression—We next analyzed the overall distribution of B cells in spleen, bone marrow, peripheral blood, and lymph nodes and found that the respective cell populations of pre-B cells (IgM+ IgD−), newly generated B cells (IgM+ IgD−), and mature recirculating B cells (IgM+ IgD+) exhibited comparable percentile representations in both SRF-deficient (CD19-Cre Srffl/fl) and control animals (Srffl/wt and CD19-Cre Srffl/wt) (data not shown). Quantification of the number of lymphocytes and CD19+ cells from bone marrow, however, showed a decrease in the overall number of CD19+ lymphocytes (Fig. 4A, left panel), which could not be attributed to the reduction of an individual B cell population (Fig. 4A, right panel). To our surprise, SRF-deficient mature B cells (CD19-Cre Srffl/fl) from spleen, lymph nodes, bone marrow, and blood displayed a lower expression of the surface molecule IgM in comparison to controls (Fig. 4B, left; shown for blood only), whereas the expression of IgD, which is cotranscribed, remained constant (Fig. 4B, right). We also observed down-regulation in the expression of CD19 in SRF-deficient B cells from peripheral blood. This is only partly caused by the hemizygosity of the CD19 allele, as shown by FACS analysis comparing B cells from CD19-Cre Srffl/fl mice with B cells from control animals expressing Cre recombinase (CD19-Cre Srffl/wt and CD19-Cre Srffl/wt) (Fig. 4C). Next, we tested the ability of SRF-deficient splenic B cells to elicit a calcium response to the F(ab′)2 fragment of anti-IgM antibodies. No differences in [Ca2+]i, between wild-type and mutant cells were observed when the splenic B cells were stimulated by suboptimal or optimal concentrations of anti-IgM antibodies (data not shown). This analysis confirms that the BCR complex on SRF-deficient B cells is capable of receiving and delivering signals. In
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Next, we examined a possible influence of SRF on the self-renewing mature CD5+ B1 B cells. Analysis of peritoneal cells from CD19-CreSrffl/fl mice revealed a substantial decrease of CD19+ IgM+CD5+ B cells in comparison to littermate controls (Srffl/fl and CD19-CreSrffl/fl) (Fig. 6). Down-regulation of SRF Target Genes in the B Cell-specific Srf Mutant—To identify SRF target genes that could account for the observed phenotypic differences, we compared the gene expression profiles of FACS-sorted splenic B cells from mutant (CD19-CreSrffl/fl) and control mice (Srffl/fl) using cDNA expression arrays (Affymetrix MOE430A GeneChip, 22690 transcripts) in two independent experiments. The microarray data were deposited in the NCBI gene expression and hybridization array data repository with assigned accession number (GEO Series Acc GSE7412). We filtered for genes whose expression was at least 1.5-fold differentially regulated. In addition to the expected down-regulation of known SRF target genes, such as the immediate early genes egr-1, junB, and c-fos and the structural genes encoding γ-actin, β-actin, and vimentin, a contribution of SRF was observed to the regulation of the accessory immunoglobulins Igα and Igβ, and CD19, which we already knew from our FACS analysis (see Fig. 4C), and the chemokine receptor CXCR4. Also, in the case of CXCR4 we were able to confirm its down-regulation on protein level by FACS analysis (Fig. 7).

DISCUSSION

Our data show substantial evidence that SRF is involved in controlling lymphocyte maturation. We deleted the conditional Srffl allele (49) using mice expressing the Cre recombinase in developing T cells (CD4-Cre) (50) or developing B cells (CD19-Cre) (51). The phenotypes of these mutant mice reveal distinct requirements for SRF in the developmental control of different lymphocytes.

T cell-specific Srf knock-out mice showed a substantial reduction in mature SP thymocytes and nearly complete absence of peripheral T cells, prohibiting a further functional analysis of this specific T cell subset. The few remaining T cells in the periphery still contained the non-recombined Srffl allele and SRF DNA-binding activity, suggesting that the residual peripheral T cells had undergone population expansion from
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The necessity of the Ras/Erk (MAPK) pathway for this process. MAPK signaling is known to trigger SRF via its TCF cofactors. Dominant interfering forms of Ras, MEK, and ERK are able to block positive selection (31, 60). This was confirmed by the phenotype of ERK deficient mice (60). The transcription factor egr-1 is targeted by activated ERK in thymocytes (61) and egr-1-deficient mice exhibit impaired positive selection (39), whereas enforced egr-1 expression promotes positive selection (62). The TCFs are known to regulate a MAPK-responsive subset of SRF target genes, including egr-1, c-fos, and Mcl-1. Recent results from Costello et al. (48) prove the TCF SAP-1 to be a direct link between ERK signaling and the transcriptional effectors of positive selection for T cells. SAP-1 is rapidly phosphorylated upon TCR activation via the ERK pathway, and SAP-1 deficiency caused impairment of egr-1 and Id-3 gene activation and decreased the amount of positively selected thymocytes. T lineage-specific deletion of Srf increased the severity of the SAP-1 phenotype leading to stronger reductions in the proportion of CD4+ SP and CD8+ SP thymocytes and peripheral T cells. This probably reflects the functional redundancy between the TCFs. Although SAP-1 was demonstrated to be the main constituent of ternary complexes in thymocytes, Elk-1 was also shown to be present (48). We demonstrated that SRF is required for T cell development following the DP stage, coinciding temporally with CD4-Cre expression and Srf deletion. However, it is likely that SRF has important functions also in the early, TCR-independent phase of T cell development, during e.g. T-lineage commitment, the transition from the CD4-CD8- DN to the CD4+CD8- DP stage, or for the survival of mature peripheral T cells. These assumptions are supported by the finding that SRF binding activity is already abundant early in thymocyte development at the DN stage (26). Moreover, studies with egr-1 knock-out mice demonstrated defective positive selection and an increased amount of DN thymocyte precursor cells (39). Also, conditional deletion of the SRF target gene Mcl-1 arrested T cell development at the DN stage, coinciding temporally with CD4-Cre expression and Srf deletion. However, it is likely that SRF has important functions also in the early, TCR-independent phase of T cell development, during e.g. T-lineage commitment, the transition from the CD4-CD8- DN to the CD4+CD8- DP stage, or for the survival of mature peripheral T cells. These assumptions are supported by the finding that SRF binding activity is already abundant early in thymocyte development at the DN stage (26). Moreover, studies with egr-1 knock-out mice demonstrated defective positive selection and an increased amount of DN thymocyte precursor cells (39). Also, conditional deletion of the SRF target gene Mcl-1 arrested T cell development at the DN...
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stage, whereas deletion in peripheral T cell populations resulted in their rapid loss (43). The effects of a SRF deficiency on the very early stages of T cell development may be addressable using Ick-Cre mice (63), permitting Cre-mediated deletion at the most immature CD44<sup>+</sup> DN stage. On the other hand, an influence of SRF on late stages of T cell development may be detectable using inducible CD44-Cre or inducible mx-Cre mouse strains (64).

B cell development in adult mice occurs in the bone marrow. Progenitor and precursor cells differentiate into immature B cells after expressing a surface immunoglobulin receptor (IgM). Newly generated B cells leave the bone marrow and some of these cells, after further positive and negative selection steps, give rise to mature B cells. Based on phenotypic, topographic, and functional characteristics, B cells are classified into at least three subsets. The heterogeneous recirculating B2 cells localize to the B-lymphoid follicles of lymph nodes and spleen, the self-renewing nonrecirculating B1B cells are enriched in the pleural and peritoneal cavities, and the mostly nonrecirculating MZB cells are enriched in the marginal zone of the spleen (65).

Our findings imply a specialized role for SRF in CD5<sup>+</sup> B1 cells and MZB cells. No obvious B cell function was described for the SRF cofactor SAP-1 (48), possibly indicating functional redundancy of different TCFs in B1 cells. These populations are substantially decreased or even lost, respectively, in the B cell-specific Srf mutant, which precludes a further functional analysis of these cell types. Surprisingly, we found SRF to be largely dispensable for the development of conventional B2 lymphocytes. These display no obvious change in BCR function, and there is no difference in the basal serum immunoglobulin concentrations in mutant animals as compared with wild types.

FACS analysis of the remaining B2 cells revealed a downregulation of the surface molecules CD19, IgM, and CXCR4. Using gene arrays we detected decreased expression of c-fos, egr-1, junB, Iga, Igβ and several genes coding for structural proteins. As IgM and IgD are transcribed as a common pre-RNA, a transcriptional change should lead to altered expression of both genes, but the level of IgD in our mutants remained unchanged. We interpret this to be due to the reduced Iga message observed in the gene array. Iga is necessary for the display of IgM and IgD on the cell surface, but IgD in contrast to IgM, can be alternatively anchored via a lipid tail in the cell membrane (66).

An interesting yet difficult to address question concerns the mechanism by which SRF promotes the development of MZB and B1-type B cells. There is distinct uncertainty in the field regarding the identity of immediate precursors to these B cell subsets and signals leading to their development. The fact that numerous mutations that modulate BCR signaling (67, 68) result in altered development of MZB or B1 cells led to the conclusion that increased BCR signaling is involved in the development of these B cell subtypes. If this were correct one would imagine an interplay between SRF signaling and BCR signaling. Hints for this were found in the observed reductions in the amount of the B cell coreceptor CD19, the surface molecule IgM, and the associated signal transducing elements Iga and Igβ in the remaining B cells from our mutants. In that respect, it is worth mentioning that CD19-deficient mice present multiple B cell defects, including a severe reduction in MZB and B1 cells (56, 69). However, we did not detect any obvious change in BCR function as determined by measuring calcium flux activation.

Our array data proved c-fos to be an SRF target gene in B cells. As overexpression of c-fos in mice elicited an increase in peritoneal B1 cells and MZB cells (35, 36), up-regulation of c-fos by SRF could be one possible mechanism to influence the size of these specific B cell subsets. Defects in cell migration might similarly account for the observed phenotypes in our B cell specific SRF mutants. Indeed, ES cells lacking SRF display impaired cell-cell interactions, disorganization of the cytoskeleton, and down-regulation of surface proteins (20). Furthermore, an influence of the actin cytoskeleton and integrin-mediated processes in lymphocyte retention and localization to the marginal zone has been shown (70, 71). Altered cytoskeletal activity in SRF deficient B cells may be indicated by the down-regulation of the actin, vimentin and vinculin genes, as detected by our array analysis. In addition, we noticed a slight decrease in the amount of beta1 and beta7 integrin (data not shown).

Decreased expression of the chemokine receptor CXCR4 could likewise contribute to the phenotypic differences seen in our SRF mutant. B cell-specific inactivation of CXCR4 affects B cell migration leading to reductions in the B1 and MZB cell compartments (72). However, a general migration defect in B cells from our mutants can be excluded, as they can be detected in the peripheral lymph nodes.

In summary, we showed that SRF is required for the development of T cells from the DP stage onward and for the development of the B1 and MZB subsets of B cells. Surprisingly, SRF deficiency does not perturb the development or survival of conventional B cells, which suggests that SRF and its cofactors mediate distinct physiological functions in different types of lymphocytes. Thus, SRF ablation does not have pleiotropic effects on the general transcription machinery in all lymphocytes, suggesting that alternative transcriptional programs exist to regulate the survival and maintenance of B2 cells. In addition, there has to be a redundancy in factors regulating known SRF-target genes like Mcl-1, which was shown to be required for the survival of all sets of B lymphocytes. However, as the CD19-Cre regulatory element used to direct Cre recombinase is not active from the beginning of B lymphocyte development one could imagine that deletion at an earlier time might result in a more severe phenotype. This hypothesis is supported by the general decrease in the number of conventional B cells seen in our mutants. Using a Cre transgenic mouse line deleting earlier in B cell development, i.e. MB1-Cre (73) should reveal additional roles of SRF in the early processes of B cell differentiation.

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