Downregulated Expression of SHP-1 in Burkitt Lymphomas and Germinal Center B Lymphocytes

By C. Charlotte Delibrias,* J. Eike Floettmann,‡ Martin Rowe,‡
and Douglas T. Fearon*

*From the *Wellcome Trust Immunology Unit, Department of Medicine, University of Cambridge School of Clinical Medicine, Cambridge CB2 2SP, United Kingdom; and ‡Department of Medicine, University of Wales, College of Medicine, Heath Park, Cardiff CF4 4XX, United Kingdom*

Summary

We wish to identify developmental changes in germinal center B cells that may contribute to their rapid growth. SHP-1 is an SH2 domain–containing phosphotyrosine phosphatase that negatively regulates activation of B cells and other cells of hematopoietic lineages. We have found that in all 13 EBV-negative and 11 EBV-positive Burkitt lymphomas with a nonlymphoblastoid phenotype, the mean concentration of SHP-1 was reduced to 5% of that of normal B and T cells. The possibility that this diminished expression of SHP-1 was related to the germinal center phenotype of Burkitt lymphomas was supported by the low to absent immunofluorescent staining for SHP-1 in germinal centers, and by the inverse relationship between the concentration of SHP-1 and the expression of the germinal center marker CD38 on purified tonsillar B cells. In CD38-high B cells, SHP-1 concentration was 20% of that of mantle zone B cells from the same donor. This reduction in SHP-1 is comparable to that of cells from motheaten viable me^v/me^v mice in which there is dysregulated, spontaneous signaling by cytokine and antigen receptors. Therefore, germinal center B cells may have a developmentally regulated, low threshold for cellular activation.

SHP-1 is a phosphotyrosine phosphatase (PTPase)[1] that is expressed mainly in cells of hematopoietic lineages. It is comprised of a phosphatase domain and two SH2 domains which bind phosphotyrosyl peptides having the consensus sequence pYXXL (1–4). Binding of phosphotyrosyl peptides to the NH_2-terminal SH2-domain relieves the catalytic site from autoinhibition by this domain, whereas the COOH-terminal SH2 domain serves only to promote attachment of the PTPase to tyrosine phosphorylated proteins (5–7). Signaling by three categories of receptors has been shown to be negatively regulated by SHP-1: receptor tyrosine kinases such as c-kit (8–10), CSF-1 receptor (11, 12), TrkA (13), and the EGF receptor (14, 15); cytokine receptors such as the IL-3 receptor (16), the interferon α/β receptor (17), and the erythropoietin receptor (18, 19); and receptor complexes of the immune system that have subunits containing the immune receptor tyrosine-based activation motif (20–27). In receptor tyrosine kinases, SHP-1 suppresses signaling by dephosphorylating the activated receptors (8–10, 12, 14, 15). Among the cytokine receptors, SHP-1 binds to phosphotyrosines of noncatalytic subunits of the receptors and dephosphorylates the autocatalytic phosphotyrosines of the associated Janus kinases (17, 19). The immune receptor tyrosine-based activation motif family of receptor complexes demonstrates a more diverse pattern for recruiting SHP-1. In T cells, SHP-1 has been reported to bind to the tyrosine kinase, ZAP-70 (20), TCR-ϵ, and CD5 (21) to inhibit signaling by the T cell receptor, whereas in NK and B cells, membrane proteins distinct from those of the activating receptor complex, the killer cell inhibitory receptor (22), FcγRIIB (23), and CD22 (24–27) bind SHP-1. Juxtapositioning of these inhibitory receptors to the activating receptors allows SHP-1 to suppress the stimulation of B and NK cells (22–24, 28).

The biological importance of SHP-1 in B cells has been exemplified by analyses of motheaten (*me*/*me*) and motheaten viable (*me^v*/*me^v*) mice in which expression of the PTPase is impaired. In contrast to the *me*/*me* mouse which has an early frameshift mutation and no detectable levels of SHP-1, the *me^v*/*me^v* mouse expresses two SHP-1 proteins that have only 10–20% normal activity (29, 30). Both strains have elevated serum levels of IgM and expansion of the B-1 subset of B cells (31) which may reflect either excessive stimulation through membrane immunoglobulin (mIg), the

---

[1] *Abbreviations used in this paper:* GC, germinal center; HEL, hen egg lysozyme; LCL, lymphoblastoid cell line; *me^v*/*me^v*, motheaten viable; mIg, membrane Ig; PTPase, phosphotyrosine phosphatase; RT, room temperature; tTA, tetracycline-controlled transactivator.

J. Exp. Med. © The Rockefeller University Press • 0022-1007/97/11/1575/09 $2.00
Volume 186, Number 9, November 3, 1997 1575–1583
http://www.jem.org

IL-5 receptor which shares a common β chain with the IL-3 receptor, or both. In a model system of mice expressing mIg specific for hen egg lysozyme (HEL) on the *me*^{v}/*me*^{v} background, there was a lower threshold for signaling through mIg (32). A similar abnormality has been observed in *CD22*$^{-/-}$ mice (33–36), consistent with CD22 inhibiting B cell activation through its recruitment of SHP-1. Interfering with the interaction of FcγRIIB and SHP-1 by deleting either protein also promotes B cell activation through mIg (32, 37) suggesting that the recruitment of SHP-1 by this receptor can suppress signaling, although the inositol polyphosphate 5-phosphatase SHIP may contribute to these inhibitory effects of FcγRIIB (38).

The pivotal role of SHP-1 in determining whether mature B cells respond to antigen led us to examine its levels during the phase of rapid, antigen-dependent expansion in the germinal center. We find that the cellular concentration of SHP-1 is reduced in both primary and transformed centroblasts to levels comparable or less than with those of *me*^{v}/*me*^{v} mice, suggesting that this developmental stage of the B cell may have hypersensitive responses to antigen or growth factors.

Materials and Methods

Cells. Cell lines were maintained in RPMI supplemented with 10% FCS, penicillin (100 U/ml), streptomycin (100 μg/ml) (GIBCO, Uxbridge, UK). Tonsillar mononuclear cells were purified by centrifugation over Ficoll–Hypaque (Pharmacia LKB Biotechnology, Uppsala, Sweden) followed by separation into high and low density lymphocytes by centrifugation through 30, 50, 55, and 60% Percoll gradient (Pharmacia LKB Biotechnology). The low density population was enriched in germinal center (GC) B cells by depleting T and follicular mantle zone B cells using anti-CD3 UCHT-1 (a gift from Dr. Claire Hivroz, Paris, France), anti-CD5 (Coulter Corp., Hialeh, Florida), anti-CD39 (Serotec Ltd., Oxford, UK) and anti-IgD (DAKO, Bucks, UK) IgG1 mAbs followed by anti–mouse IgG-coated magnetic beads (Dynabeads; Dynal, Oslo, Norway). GC cells were then purified by sorting with a FACSVantage® (Becton Dickinson, Oxford, UK) after labeling cells with FITC-conjugated anti-CD19 (Coulter Corp.) and PE-conjugated anti-CD38 mAbs (Becton Dickinson). In some experiments, enriched GC cells were labeled with the anti-CD77 IgM rat mAb (Immunotech, Marseilles, France) followed by FITC-conjugated goat anti–rat IgM Ab (The Binding Site, Birmingham, UK) and with PE-conjugated anti-CD38 IgG1 mAb in the presence of an excess of an irrelevant IgG1 mAb, MOPC21. Cells were sorted into CD38-positive, CD77-positive (centroblasts), and CD38-positive, CD77-negative (centrocytes) subpopulations. Resting CD19-positive, CD38-negative mantle zone B cells were purified from the high density fraction by sorting. To obtain memory B lymphocytes, cells from the high density fraction were depleted in T and activated B cells by using anti-CD3, anti-CD38 Ab (Becton Dickinson) and anti–mouse IgG-coated magnetic beads. The resulting population, which was 97% CD19-positive, was stained with an FITC-conjugated goat anti–human IgG γ chain–specific (Sigma Chemical Co., Poole, UK) and IgG-positive cells were purified by sorting.

Assay of SHP-1. Cell lysates were prepared at 4°C in buffer containing 1% NP-40, 50 mM Tris/HCl, pH 7.5, 10 mM EDTA, 80 mM KCl, and 50 μM PMSF, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 1 μg/ml antipain, 1 μg/ml pepstatin A, 1 μg/ml chymostatin (all from Sigma Chemical Co.). The particulate fraction was removed by centrifugation at 13,000 *g*, and protein concentration in the soluble lysate was assayed by the BCA protein assay kit (Pierce, Chester, UK).

Affinity-purified anti-SHP-1 antibody was developed by immunizing rabbits with a glutathione-*S* transferase (GST)-SHP-1 fusion protein (3). Immune immunoglobulin was adsorbed to and eluted from immobilized recombinant SHP-1 that had been rendered free of GST by thrombin cleavage of the GST-SHP-1 fusion protein. For the ELISA assay of SHP-1 in cell lysates, the anti-SHP-1 antibody was coated onto 96-well Nunc immunoplates (GIBCO) at 2.5 μg/ml in 50 mM carbonate buffer (pH 9.6) at 4°C overnight. Plates were washed in PBS containing 0.05% Tween-20 (vol/vol), blocked with 1% BSA in the same buffer for 2 h at room temperature (RT), and sequentially incubated for 1 h at RT with serial dilutions of cell lysate, biotinylated rabbit anti-SHP-1 at 1 μg/ml, and horseradish peroxidase–conjugated streptavidin (Pierce). Plates were read at OD of 450 nm 30 min after addition of *o*-phenylenediamine (OPD) as the substrate (Sigma Chemical Co.). A standard curve was established using recombinant SHP-1 free of GST. The SHP-1 concentration in cells was calculated as the ratio between SHP-1 concentration and total protein concentration, and expressed as percent SHP − 1/total cellular protein.

For Western blot analysis, proteins from total cell lysates (150 μg/lane) were separated by 10% SDS-PAGE, electrotransferred to nitrocellulose membrane, blocked with 1% fatty acid–poor BSA (Calbiochem, Nottingham, UK), and immunoblotted with the affinity-purified rabbit anti-SHP-1 antibody followed by horseradish radish–conjugated mouse anti–rabbit IgG (Jackson Immunoresearch, Westgrove, PA). The blots were visualized with the enhanced chemiluminescence detection system (Amersham, Little Chalfont, UK).

For functional assay of SHP-1, 5 × 10^6 cells were lysed in 1 ml of NP-40 lysis buffer and 2 μg of affinity-purified anti-SHP-1 was added for 60 min at 4°C. Immune complexes were absorbed with protein A (Pierce) for 60 min at 4°C and washed four times with phosphatase assay buffer (20 mM imidazole, pH 7.0, 0.2% β mercaptoethanol). The synthetic peptide substrate Raytide (Calbiochem), was labeled using γ-[^{32}P]ATP (Amersham) and p43 abl kinase (Calbiochem; references 1, 39). The activity of SHP-1 in the immune complex was assayed after addition of an activating phosphotyrosyl peptide (1 μg/ml) corresponding to Y843 of the cytoplasmic domain of human CD22, and is expressed as cpm of [^{32}P]O_4 released from the Raytide.

Transfections. The EBV-negative Burkitt cell line DG75 was stably transfected with a modified tetracycline-controlled transactivator (tTA)-dependent expression system using the plasmids, pJEF3 encoding tTA, and the expression vector PJEF4 (40). Three constructs were prepared with the latter vector: PJEF4–SHP-1 and PJEF4–αSHP-1 by inserting SHP-1 cDNA (3) into the EcoRI cloning site in the sense and antisense orientations, respectively, and PJEF4–SHP-1(C453S) in which the codon for C453 in the enzyme active site was mutated to S (USE mutagenesis kit, Pharmacia). Cells were transfected by electroporation at 250 V using a Bio-Rad (Hercules, CA) gene pulser. A stable line, DG75tTA, expressing tTA, was first established and selected on the basis of a high transactivator expression when transiently supertransfected with a tTA-dependent luciferase reporter unit (40). This line was subsequently stably cotransfected in the presence of tetracycline (1 μg/ml) with the three SHP-1 plasmids. Hygromycin- and neomycin-resistant clones were assayed for SHP-1 expression by

ELISA and Western blot 24 to 48 h after withdrawal of tetracycline.

Immunofluorescent Staining of Tonsillar Sections. Tonsils taken from patients during routine tonsillectomy were flash frozen in Cryo-M-Bed embedding media (Bright Instrument Company Ltd., Huntington, UK). Serial, 5-μm–thick frozen sections were cut and mounted onto poly-l-lysine–coated slides, air dried, permeabilized, and fixed in cold acetone/methanol (50/50% vol/vol) for 15 min, washed in Tris-buffered saline, blocked in 10% FCS, and incubated with affinity-purified rabbit anti-SHP-1 or rabbit anti-GST, and either anti-CD38 or anti-CD19 mAbs for 1 h at RT, followed by FITC-conjugated F(ab′)$_2$ goat anti–rabbit Ig (Jackson Immunoresearch) and TRITC-conjugated goat F(ab′)$_2$ anti–mouse Ig (Sigma Chemical Co.). The stained sections were examined by fluorescent microscopy. Images were captured using smartCapture software (Digital Scientific) from a cooled CCD camera (Photometrics KAF1400) mounted on a Zeiss Axioskop microscope equipped with an automated filter wheel, triple band-pass filter and a 20× objective (total magnification 200×). TRITC and FITC images were captured separately as black and white 24-bit images, merged, and displayed as a final color 24-bit picture. All image processing was performed on a Macintosh Quadra 840AV using IPLab Software.

Results

Levels of Expression of SHP-1 among Lymphocytes. We initially compared the amount of SHP-1 in six human B cell lines by Western blot analysis and found that the PTPase was expressed at different levels among these lines (Fig. 1). Although SHP-1 was easily detected in lysates from two EBV-immortalized lymphoblastoid cell lines (LCLs), WW2LCL and JY, it was reduced in three of three Burkitt lymphomas: Ramos, Daudi, and WW2BL. BJAB, a B lymphoma line morphologically resembling EBV-negative Burkitt lymphoma but lacking the characteristic chromosomal translocation that involves *c-myc* (41), expressed higher levels of SHP-1. To quantitatively assay SHP-1 concentration in cell lysates, we developed an ELISA using an affinity-purified polyclonal rabbit antibody specific for SHP-1 as both the capture and detecting antibody, and recombinant SHP-1 as the standard. The measurement of SHP-1 confirmed the results of the semiquantitative Western blots, indicating that in the three Burkitt lymphomas with *c-myc* translocations, the SHP-1 intracellular concentrations were reduced relative to the levels in the EBV-immortalized lines (Fig. 1).

We assayed the concentration of SHP-1 in a large panel of cells, including primary T and B cells, granulocytes, EBV-immortalized LCLs from healthy individuals, EBV-negative and -positive Burkitt lymphomas, and LCLs established with normal B cells from patients with Burkitt lymphoma (Fig. 2). The concentration of SHP-1 in normal primary T and B cells purified from tonsils of five individuals ranged between 0.04 and 0.14% (mean = 0.085%) of total cellular protein (Fig. 2). Activation of B cells in vitro with pokeweed mitogen did not alter the levels of SHP-1 (data not shown). Granulocytes purified from the peripheral blood of five individuals had similar concentrations of SHP-1 (mean = 0.062% of total cellular protein), as did eight LCL lines from normal individuals (mean = 0.070% of total cellular protein). In 13 of 13 EBV-negative Burkitt lymphoma lines, SHP-1 levels were reduced by one to three orders of magnitude relative to the levels in normal lymphocytes (mean = 0.004% of total cellular protein). Group I/II EBV-positive Burkitt lines, which have retained the original tumor biopsy phenotype and which morphologically resemble EBV-negative lines, had comparably reduced concentrations of SHP-1 (mean = 0.005% of total cellular protein). In contrast, the levels of SHP-1 were normal in four of four group III EBV-positive Burkitt lymphomas (mean = 0.077% of total cellular protein) which resemble LCLs morphologically, by surface phenotype, and by EBV gene expression (42). In the seven Burkitt lines that had paired LCLs established from normal B cells of these individuals, the lymphomas had reduced levels of SHP-1 (mean = 0.003% of total cellular protein), whereas the LCLs (mean = 0.094% of total cellular protein) did not differ from normal B cells or from the cell lines established from healthy individuals.

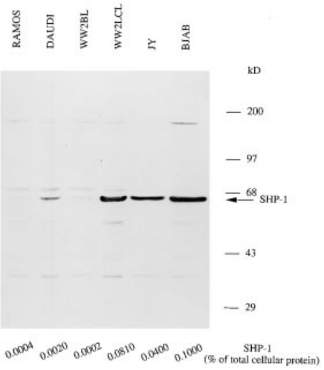

Figure 1. Western blot analysis of SHP-1 in lysates from the Burkitt lymphomas, Ramos, Daudi and WW2BL, the LCLs, WW2LCL and JY, and the B lymphoma BJAB. The numbers at the bottom of the gel represent the concentration of SHP-1 in the cellular lysates as determined by ELISA.

The apparent reversion to normal of SHP-1 levels in EBV-infected Burkitt lymphomas in association with an LCL phenotype suggested that the expression of the PTPase could be modulated. This possibility was demonstrated by stimulating the EBV-negative Burkitt line, Ramos, and the EBV-positive lines Daudi and WW2BL, with either phorbol ester (PMA) or polyclonal antibody to IgM. In Ramos and Daudi, both treatments caused time-dependent increases of 10–50-fold in the cellular concentration of SHP-1, whereas in the WW2BL line, only PMA was effective because these cells do not express mIg (Fig. 3). Additional experiments with the Ramos line showed that the surface markers characteristic of Burkitt lymphomas, CD38 and CD77, were unchanged after mIgM ligation, but that the ratio of CD19 to CD22 was inverted due to an increase in CD22 and a decrease in cell surface CD19 (data not shown). As previously reported (43), stimulation of all three Burkitt lymphomas caused apoptosis (not shown).

Transfection of a Burkitt Lymphoma Line with Plasmids Permitting Tetracycline-regulated Expression of SHP-1. To investigate the biological effects of normalizing the cellular concentration of SHP-1 by a means that is independent of cellular stimulation, we stably transfected the EBV-negative Burkitt line, DG75, with plasmids directing tetracycline-suppressible expression of wild-type SHP-1, SHP-1 in which the active site cysteine is replaced with serine, and SHP-1 in the

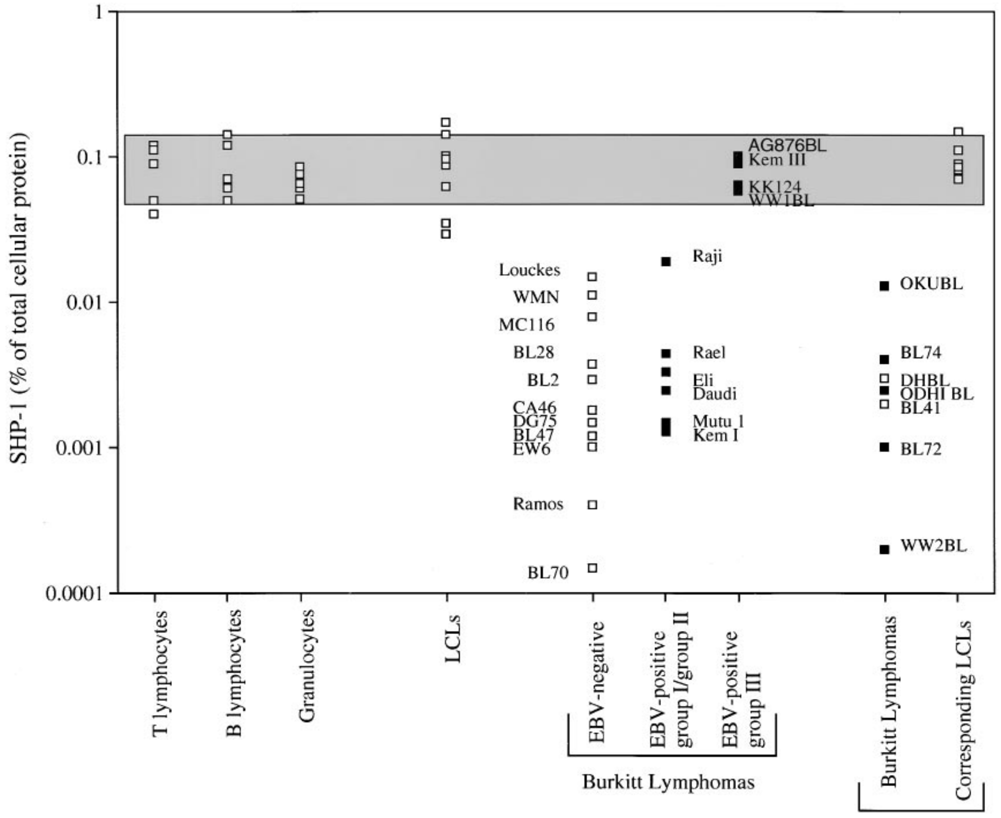

Figure 2. Concentration of SHP-1 in primary and transformed human cells as determined by ELISA. The closed symbols designate EBV-positive Burkitt lymphomas. The shaded area represents the mean ± 1 SD of the SHP-1 levels in normal B and T cells.

antisense orientation. Removal of tetracycline from the medium led to a 10–40-fold increase over 48 h in the concentration of wild-type SHP-1 in the clone S9.26, and of SHP-1–inactive mutant in the CS2.30 clone; no increase occurred with the AS14.28 clone transfected with the antisense construct (Fig. 4). Analysis by Western blot indicated that both forms of SHP-1 comigrated with the PTPase expressed by LCLs (data not shown). Assay of phosphotyrosine phosphatase activity revealed that immunoprecipitates of SHP-1 from uninduced and induced S9.26 clones released 112 cpm and 2,022 cpm, respectively, from $[^{32}P]O_4$-labeled Raytide. This level of PTPase activity in the lysate of induced S9.26 cells was comparable to that of the WW2LCL line, which was 2,144 cpm. The CS2.30 clone, whether grown in the presence (128 cpm) or absence (148 cpm) of tetracycline, showed little SHP-1 activity.

We examined the effects of elevating SHP-1 on the membrane expression of IgM, apoptosis, and DNA synthesis. The induction of SHP-1 expression after removal of tetracycline from S9.26 cells was associated with twofold increase in mIgM, as assessed by flow cytometry of cells stained with monoclonal anti-IgM (Fig. 4). In three replicate experiments with this clone, similar increases of two- to threefold in mIgM were observed, and inducibly expressing SHP-1 in an additional, independent clone was also associated with increased mIgM. There was no change in the levels of three other membrane proteins, CD19, CD22, and CD38 (not shown). This response to SHP-1 required its phosphatase activity because comparable increases in the protein concentration of the SHP-1–inactive mutant in clone CS2.30 were not accompanied by changes in mIgM expression (Fig. 4). The clone expressing the antisense plas-

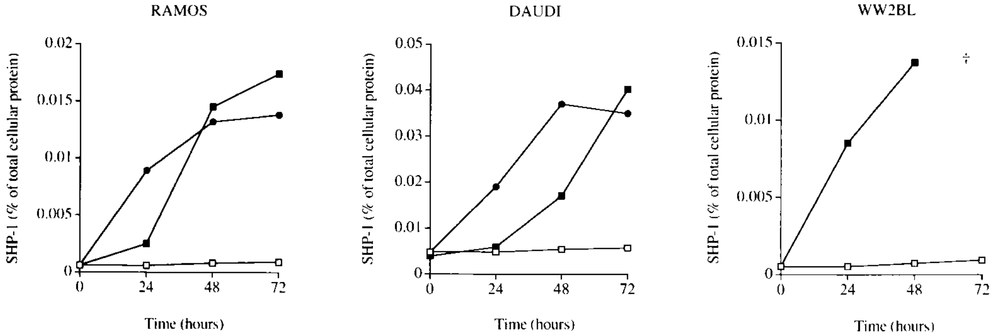

Figure 3. Kinetics of upregulation of SHP-1 expression in untreated Burkitt lymphomas (*open squares*), in lymphomas stimulated with 20 ng/ml PMA (*closed squares*), or with 5 μg/ml polyclonal goat $F(ab')_2$ anti-IgM (*closed circles*). †>70% of WW2BL cells had undergone apoptosis after 72 h of PMA treatment.

mid also showed no change in mIgM. We also examined the effect of elevating SHP-1 on the Ca^{2+} response induced by ligating mIgM, but found no change, perhaps because the level of CD22 is only 2% that of primary, mantle zone B cells, which would prevent the recruitment of SHP-1 to the antigen receptor complex (24, 33–36).

In contrast to this effect of restoring SHP-1 levels on mIgM, there was no alteration in the frequency of cells undergoing apoptosis during culture in 10% FCS when assayed by staining with annexin V and propidium iodide (not shown). Furthermore, the incorporation of $[^3H]$thymidine by cells which had normalized their SHP-1 level was the same as that of cells in which low SHP-1 was maintained by the presence of tetracycline (not shown). This clone did undergo apoptosis in response to ligating mIgM; 37% of cells were annexin V–positive, propidium iodide–negative with anti-IgM at 48 h, compared to 12% without anti-IgM. At least two other experiments with the S9.26 clone also showed no effect of induced SHP-1 on apoptosis and DNA replication. In addition, similar results were observed with three other clones. Therefore, low SHP-1 in the DG75 Burkitt lymphoma contributes to the diminished expression

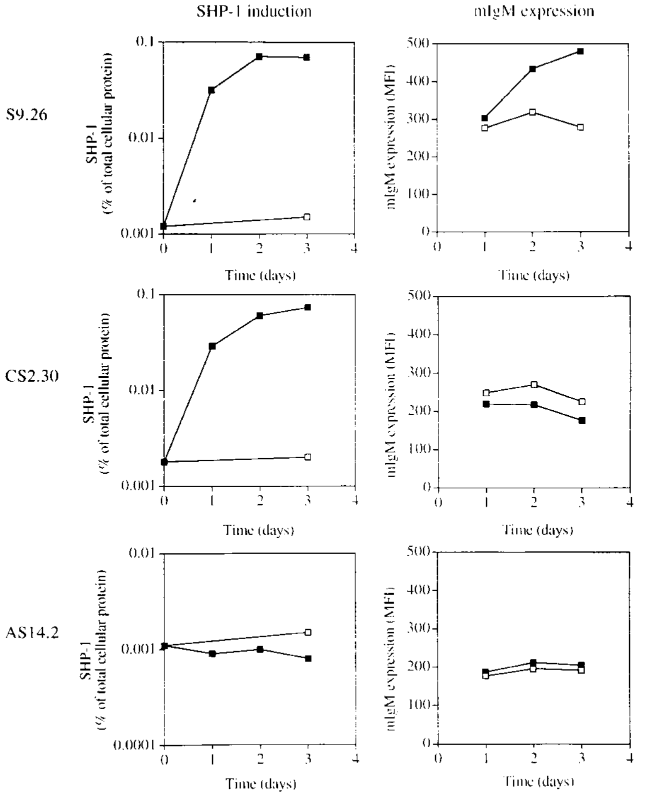

Figure 4. Effects of inducing SHP-1 on the quantitative expression of mIgM in three DG75 Burkitt lymphoma clones transfected with tTA-regulated plasmids encoding wild-type SHP-1 (S9.26), the phosphatase-inactive C453S mutant SHP-1 (CS2.30), and SHP-1 cDNA in the antisense orientation (AS14.2), respectively. The coexpression of a plasmid for tTA enables tetracycline to suppress transcription of the SHP-1 constructs. After culture of cells in the presence (*open symbols*) or absence (*closed symbols*) of tetracycline, SHP-1 levels were measured by ELISA and mIgM expression by FACS® analysis of cells stained with a monoclonal anti-IgM antibody.

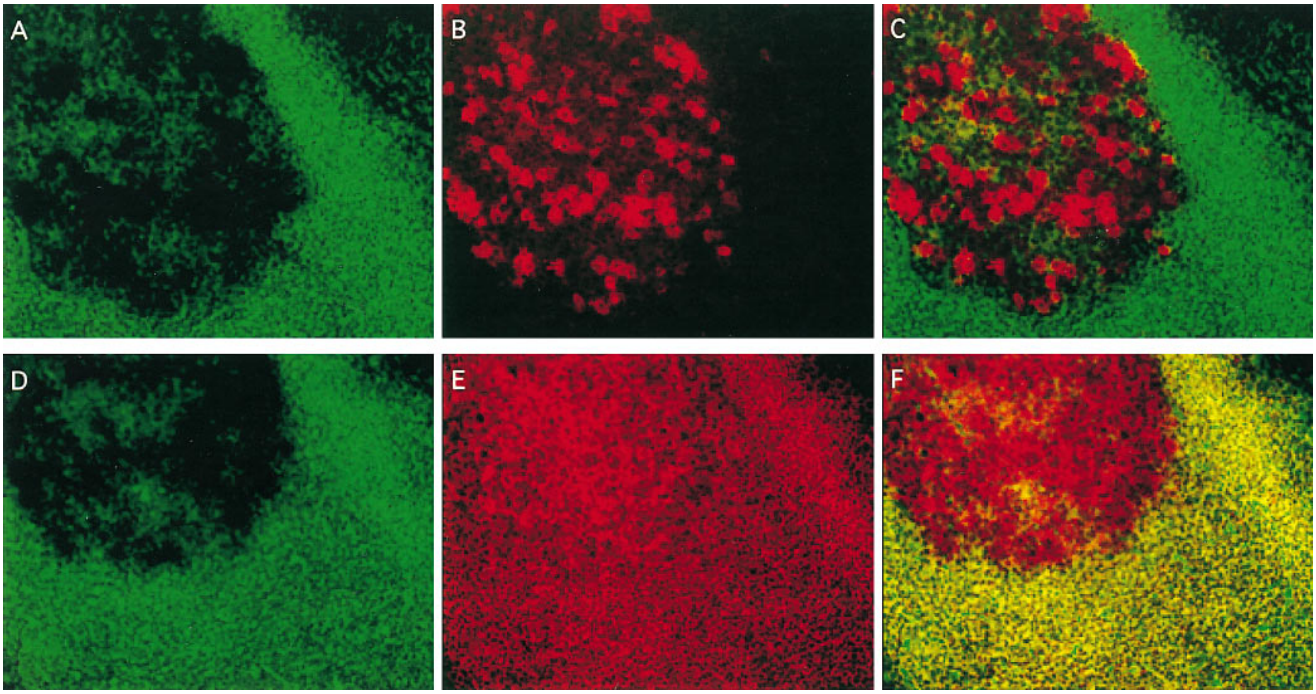

Figure 5. Analysis of SHP-1 expression in tonsillar GCs by immunofluorescent microscopy. *A–C* are photomicrographs of a single section stained with FITC-conjugated anti-SHP-1 (*A*), TRITC-conjugated anti-CD38 (*B*), and a computor generated superimposition of these two images (*C*). *D–F* are photomicrographs of an adjacent section stained with FITC-conjugated anti-SHP-1 (*D*), TRITC-conjugated anti-CD19 (*E*), and a computer generated superimposition of these two images (*F*). Magnification is 200.

of mIgM on these cells, but apparently not to their growth or viability in tissue culture.

Diminished Expression of SHP-1 in GC B Cells. Burkitt lymphomas exhibit a centroblast phenotype (44) and can be induced to mutate their immunoglobulin genes in vitro (45), the defining functional characteristic of the GC B cell. Thus, the low abundance of SHP-1 in these cell lines could reflect the phenotype of GC B cells rather than being a consequence of transformation. To investigate the expression of SHP-1 in normal GC cells, frozen tonsil sections were stained for SHP-1 and the GC B cell membrane protein, CD38, or for SHP-1 and the pan–B cell membrane protein, CD19. Cells in the mantle zone stained brightly for SHP-1, but the PTPase was diminished or absent in the CD38-positive GC cells (Fig. 5). Superimposition of the two images confirmed that no cells expressing high CD38 were positive for SHP-1. Staining of an adjacent section for SHP-1 and CD19 demonstrated that CD19 was distributed equally among mantle zone and GC B cells, but again that SHP-1 was predominantly localized to mantle zone B cells.

We also quantitated SHP-1 expression by ELISA in purified GC B cells. Total CD38- and CD19-positive B cells that had been purified from five tonsils had SHP-1 concentration that ranged from 31 to 36% (mean 33%) of the concentration in CD38-negative, CD19-positive cells. GC B cells appeared to vary according to the intensity with which they stained for CD38 (Fig. 5). Therefore, we sorted GC B cells into CD38-high and -low populations, and compared their levels of SHP-1 to the levels in CD38-negative B cells. There was an inverse relationship between CD38 expression and SHP-1 content, with the highest CD38 expressing cells having a SHP-1 level that was only 20% that of CD38-negative cells (Fig. 6). CD38-positive B cells were also sorted on the basis of CD77 expression and assayed for SHP-1; CD77-positive and -negative GC B cells had identical and diminished levels of SHP-1, indicating that both centroblasts and centrocytes downregulate the PTPase (data not shown). In two of two experiments, CD38-negative, IgG-positive, CD19-positive B cells had the same concentration of SHP-1 as did resting, CD38-negative, CD19-positive B cells purified from the same donors (data not shown), suggesting that SHP-1 levels return to normal when GC cells differentiate into memory B cells.

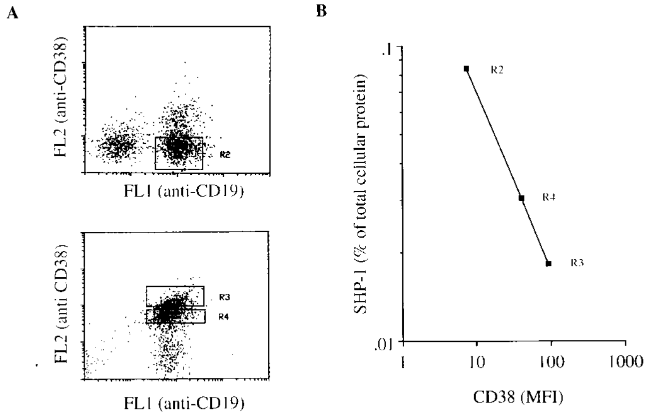

Figure 6. Inverse correlation between the levels of SHP-1 and CD38 expression in GC B cells. (*A*) CD19-positive, CD38-negative B cells (R2) were purified by sorting from high density tonsillar B cells (*top*). CD38-high (R3) and CD38-low (R4) B cells were purified by sorting from low density, CD39-negative tonsillar B cells (*bottom*). (*B*) The expression of SHP-1 and CD38 in the R2, R3, and R4 subpopulations of sorted tonsillar B cells was determined by ELISA and flow cytometry, respectively.

Discussion

We have shown that the cellular concentration of SHP-1 is diminished in primary and transformed B cells exhibiting a GC phenotype, and in B cells anatomically residing in the GC. The level to which SHP-1 is reduced in these cells is comparable to the reduction observed in hematopoietic cells of the me^v/me^v mouse in which there is dysregulated signaling through all three classes of receptors that are potentially inhibited by this PTPase (29, 30). The return of SHP-1 levels to normal in the memory B cell indicates that this suppression of SHP-1 is characteristic for the centroblast/centrocyte, perhaps reflecting a requirement at this stage of B cell development for a lower threshold for signaling via certain cytokine receptors and mIg.

These studies were initiated by the finding of low SHP-1 in all EBV-negative Burkitt lymphomas and all EBV-positive Burkitt lymphomas that have retained the original tumor biopsy phenotype, which led us to consider the possibility that relief from the inhibitory function of this PTPase may contribute to the growth or viability of these tumors. The constitutive expression of *c-myc* alone is associated with the induction of p53, cell cycle arrest, and apoptosis (46–48). These effects can be blocked by signaling through growth factor receptors that are subject to inhibition by SHP-1 (46, 49). Thus, we considered the possibility that low SHP-1 in Burkitt lymphomas might have permitted spontaneous signaling through a cytokine receptor that rescued cells from c-myc–induced apoptosis. However, we were not able to demonstrate that normalizing SHP-1 levels in the DG75 Burkitt lymphoma altered growth or viability, even in the presence of reduced concentrations of FCS (not shown). We cannot exclude the possibility that this line had accumulated additional mutations during its long-term tissue culture which had rendered it insensitive to SHP-1.

A biological effect of inducing SHP-1 was observed, which was an increase in mIgM expression in the DG75 clone expressing active PTPase, but not in the clone expressing inactive enzyme (Fig. 4). Low SHP-1 in the me^v/me^v mouse is also associated with low mIgM expression (32). It was suggested that this change was caused by spontaneous signaling through the dysregulated antigen receptor, by analogy to the diminished mIgM in HEL, anti-HEL double transgenic mice (50). We have not determined if the effects of elevating SHP-1 in the Burkitt lymphoma reflects decreased catabolism of mIgM or its increased biosynthesis, although the 24-h delay in mIgM expression, relative to induced SHP-1, would be consistent with enhanced synthesis.

That SHP-1 levels may vary with stages of cellular differentiation has also been shown in studies finding that PMA induction of HL-60 cells to a more macrophage-like phenotype was accompanied by a two- to threefold increase in the concentration of the PTPase (51, 52). However, our study is the first to demonstrate a reversible downregulation of SHP-1 expression coincident with a specific phase of cellular differentiation. Low SHP-1 may facilitate the signals required for GC clonal expansion, isotype switching, hypermutation, and selection for high affinity memory B cells. In this respect, it is interesting that two receptors that induce differentiation of primary B cells in vitro into CD38-positive cells, mIg, and the type I interferon receptor, are both regulated by SHP-1 (53). Future studies should determine whether these or other receptors are the beneficiaries of this developmentally regulated release from inhibition by this PTPase.

C.C. Delibrias was a recipient of a postdoctoral fellowship from EEC, Human Capital and Mobility Programme, and of a grant from the Leukaemia Research Fund. J.E. Floettmann was supported by a grant from the Welsh Scheme for the Development of Health and Social Research. M. Rowe was supported by the Wellcome Trust. D.T. Fearon is a Principal Research Fellow of the Wellcome Trust.

Address correspondence to Douglas T. Fearon, Wellcome Trust Immunology Unit, Department of Medicine, University of Cambridge School of Clinical Medicine, Cambridge CB2 2SP, UK. Phone: 44-1223-330-528; FAX: 44-1223-336-815; E-mail, dtf1000@cus.cam.ac.uk

Received for publication 10 July 1997 and in revised form 13 August 1997.

References

1. Shen, S.H., L. Bastien, B.I. Posner, and P. Chretien. 1991. A protein-tyrosine phosphatase with sequence similarity to the SH2 domain of the protein-tyrosine kinases. *Nature (Lond.)*. 352:736–739.
2. Yi, T., J.L. Cleveland, and J.N. Ihle. 1992. Protein tyrosine phosphatase containing SH2 domain: characterization, preferential expression in hematopoietic cells, and localization to human chromosome 12p12-p13. *J. Exp. Med.* 12:836–846.
3. Plutzky, J., B.G. Neel, and R.D. Rosenberg. 1992. Isolation of a src homology 2–containing tyrosine phosphatase. *Proc. Natl. Acad. Sci. USA*. 89:1123–1127.
4. Matthews, R.J., D.B. Bowne, E. Flores, and M.L. Thomas. 1992. Characterization of hematopoietic intracellular protein tyrosine phosphatases: description of a phosphatase containing an SH2 domain and another enriched in proline–, glutamic acid–, serine–, and threonine–rich sequences. *Mol. Cell. Biol.* 12:2396–2405.
5. Townley, R., S.-H. Shaen, D. Banville, and C. Ramachandran. 1993. Inhibition of the activity of protein tyrosine phosphatase 1C by its SH2 domains. *Biochemistry*. 32:13414–13418.
6. Pregel M.J., S.H. Shen, and A.C. Storer. 1995. Regulation of protein phosphatase 1C: opposing effects of the two src homology 2 domains. *Protein Eng.* 8:1309–1316.
7. Pei, D., J. Wang, and C.T. Walsh. 1996. Differential functions of the two Src homology 2 domain in protein tyrosine phosphatase SH-PTP1. *Proc. Natl. Acad. Sci. USA*. 93:1141–1145.
8. Yi, T., and J.N. Ihle. 1993. Association of hematopoietic cell phosphatase with c-kit after stimulation with c-kit ligand. *Mol. Cell. Biol.* 13:3350–3358.
9. Lorenz, U., A.D. Bergemann, H.N. Steinberg, J.G. Flanagan, X. Li, S.J. Galli, and B.G. Neel. 1996. Genetic analysis reveals cell type–specific regulation of receptor tyrosine kinase c-kit by the protein tyrosine phosphatase SHP1. *J. Exp. Med.* 184:1111–1126.
10. Paulson, R.F., S. Vesely, K.A. Siminovitch, and A. Bernstein. 1996. Signalling by the W/Kit receptor tyrosine kinase is negatively regulated *in vivo* by the protein tyrosine phosphatase Shp1. *Nat. Genet.* 13:309–315.
11. Yeung, Y.-G., K.L. Berg, F.J. Pixley, R.H. Angeletti, and E.R. Stanley. 1992. Protein tyrosine phosphatase–1C is rapidly phosphorylated on tyrosine in macrophages in response to colony stimulating factor–1. *J. Biol. Chem.* 267:23447–23450.
12. Chen, H.E., S. Chang, T. Trub, and B.G. Neel. 1996. Regulation of colony-stimulating factor 1 receptor signaling by the SH2 domain–containing tyrosine phosphatase SHPTP1. *Mol. Cell. Biol.* 16:3685–3697.
13. Vambutas, V., D.R. Kaplan, M.A. Sells, and J. Chernoff. 1995. Nerve growth factor stimulates tyrosine phosphorylation and activation of src homology–containing protein tyrosine phosphatase 1 in PC12 cells. *J. Biol. Chem.* 270:25629–25633.
14. Vogel, W., R. Lammers, J. Huang, and A. Ullrich. 1993. Activation of a phosphotyrosine phosphatase by tyrosine phosphorylation. *Science (Wash. DC)*. 259:1611–1614.
15. Tomic, S., U. Greiser, R. Lammers, A. Kharitonenkov, E. Imyanitov, A. Ulrich, and F.D. Bohmer. 1995. Association of SH2 domain protein tyrosine phosphatases with the epidermal growth factor receptor in human tumor cells. *J. Biol. Chem.* 270:21277–21284.
16. Yi, T., A.L.-F. Mui, G. Krystal, and J.N. Ihle. 1993. Hematopoietic cell phosphatase associates with the interleukin-3 (IL-3) receptor β chain and down-regulates IL-3–induced tyrosine phosphorylation and mitogenesis. *Mol. Cell. Biol.* 13: 7577–7586.
17. David, M., H.E. Chen, S. Goelz, A.C. Larner, and B.G. Neel. 1995. Differential regulation of the alpha/beta interferon-stimulated Jak/Stat pathway by the SH2 domain–containing tyrosine phosphatase SHPTP1. *Mol. Cell. Biol.* 15:7050–7058.
18. Yi, T., J. Zhang, O. Miura, and J.N. Ihle. 1995. Hematopoietic cell phosphatase associates with erythropoietin (Epo) receptor after Epo-induced receptor tyrosine phosphorylation:

identification of potential binding sites. *Blood.* 85:87–95.
19. Klingmuller, U., U. Lorenz, L.C. Cantley, B.G. Neel, and H.F. Lodish. 1995. Specific recruitment of SH-PTP1 to the erythropoietin receptor causes inactivation of JAK2 and termination of proliferative signals. *Cell.* 80:729–738.
20. Plas, D., R. Johnson, J.T. Pingel, R.J. Matthews, M. Dalton, G. Roy, A.C. Chan, and M.L. Thomas. 1996. Direct regulation of ZAP-70 by SHP-1 in T cell antigen receptor signaling. *Science (Wash. DC).* 272:1173–1176.
21. Pani, G., K.-D. Fischer, I. Mlinaric-Rascan, and K.A. Siminovitch. 1996. Signaling capacity of the T cell antigen receptor is negatively regulated by the PTP1C tyrosine phosphatase. *J. Exp. Med.* 184:839–852.
22. Burshtyn, D.N., A.M. Scharenberg, N. Wagtmann, S. Rajagopalan, K. Berrada, T. Yi, J.-P.J. Kinet, and E.O. Long. 1996. Recruitment of tyrosine phosphatase HCP by the killer cell inhibitory receptor. *Immunity.* 4:77–85.
23. D'Ambrosio, D., K.L. Hippen, S.A. Minskoff, I. Mellman, G. Giovani, K.A. Siminovitch, and J.C. Cambier. 1995. Recruitment and activation of PTP1C in negative regulation of antigen receptor signaling by FcγRIIB1. *Science (Wash. DC).* 268:293–297.
24. Doody, G.M., L.B. Justement, C.C. Delibrias, R.J. Matthews, J. Lin, M.L. Thomas, and D.T. Fearon. 1995. A role in B cell activation for CD22 and the protein tyrosine phosphatase SHP. *Science (Wash. DC).* 269:242–244.
25. Lankester, A.C., G.M.W. Schijnindel, and R.A.W. Van Lier. 1995. Hematopoietic cell phosphatase is recruited to CD22 following B cell antigen receptor ligation. *J. Biol. Chem.* 270: 20305–20308.
26. Campbell, M.A., and N.R. Klinman. 1995. Phosphotyrosine-dependent association between CD22 and the protein tyrosine phosphatase 1 C. *Eur. J. Immunol.* 25:1573–1579.
27. Law, C.-L., S.P. Sidorenko, K.A. Chandran, Z. Zhao, S.-H. Shen, E.H. Fischer, and E.A. Clark. 1996. CD22 associates with protein tyrosine phosphatase 1C, syk, and phospholipase C-γ1 upon B cell activation. *J. Exp. Med.* 183:547–560.
28. Tooze, R.M., G.M. Doody, and D.T. Fearon. 1997. Counterregulation by the coreceptors CD19 and CD22 of MAP kinase activation by membrane immunoglobulin. *Immunity.* 7:1–20.
29. Shultz, L.D., P.A. Schweitzer, T.V. Rajan, T. Yi, J.N. Ihle, R.J. Matthews, M.L. Thomas, and D.R. Beier. 1993. Mutations at the murine motheaten locus are within the hematopoietic cell protein-tyrosine phophatase (Hcph) gene. *Cell.* 73:1445–1454.
30. Kozlowski, M., I. Mlinaric-Rascan, G.-S. Feng, R. Shen, T. Pawson, and K.A. Siminovitch. 1993. Expression and catalytic activity of the tyrosine phosphatase PTP1C is severely impaired in motheaten and viable motheaten mice. *J. Exp. Med.* 178:2157–2163.
31. Sidman, C.L., L.D. Shultz, R.R. Hardy, K. Hayakawa, and L.A. Herzenberg. 1986. Production of immunoglobulin isotypes by Ly-1$^+$ B cells in viable motheaten and normal mice. *Science (Wash. DC).* 232:1423–1425.
32. Cyster, J.G., and C.C. Goodnow. 1995. Protein tyrosine phosphatase 1C negatively regulates antigen receptor signaling in B lymphocytes and determines threshold for negative selection. *Immunity.* 2:13–24.
33. O'Keefe, T.L., G.T. Williams, S.L. Davies, and M.S. Neuberger. 1996. Hyperresponsive B cells in CD22-deficient mice. *Science (Wash. DC).* 275:798–801.
34. Otipoby, K.L., K.B. Andersson, K.E. Draves, S.J. Klaus, A.G. Farr, J.D. Kerner, R.M. Perlmutter, C.-L. Law, and E.A. Clark. 1996. CD22 regulates thymus-independent responses and the lifespan of B cells. *Nature (Lond.).* 384:634–637.
35. Nitschke, L., R. Carsetti, B. Ocker, G. Kohler, and M.C. Lamers. 1997. CD22 is a negative regulator of B-cell receptor signalling. *Curr. Biol.* 7:133–143.
36. Sato, S., A.S. Miller, M. Inaoki, C.B. Bock, P.J. Jansen, M.L. K. Tang, and T.F. Tedder. 1996. CD22 is both a positive and negative regulator of B lymphocyte antigen receptor signal transduction: altered signaling in CD22-deficient mice. *Immunity.* 5:551–562.
37. Takai, T., M. Ono, M. Hikida, H. Ohmori, and J.V. Ravetch. 1996. Augmented humoral and anaphylactic responses in Fc γ RII-deficient mice. *Nature (Lond.).* 379:346–349.
38. Ono, M., S. Bolland, P. Tempst, and J.V. Ravetch. 1996. Role of the inositol phosphatase SHIP in negative regulation of the immune system by the receptor FcγRIIB. *Nature (Lond.).* 383:263–266.
39. Streuli, M., N.X. Krueger, T. Thai, M. Tang, and H. Saito. 1990. Distinct functional roles of the two intracellular phosphatase like domains of the receptor-linked protein tyrosine phosphatase LCA and LAR. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:2399–2407.
40. Floettmann, J.E., K. Ward, A.B. Rickinson, and M. Rowe. 1996. Cytostatic effect of Epstein-Barr virus latent membrane protein–1 analyzed using tetracycline-regulated expression in B cell lines. *Virology.* 223:29–40.
41. Menezes, J., W. Leibold, G. Klein, and G. Clements. 1975. Establishment and characterization of an EBV-negative B-lymphoma line, BJAB, from an exceptional EBV-genome–negative African Burkitt Lymphoma. *Biomedicine (Paris).* 22:276–284.
42. Rowe, M., D.T. Rowe, C.D. Gregory, L.S. Young, P.J. Farrell, H. Rupani, and A.B. Rickinson. 1987. Differences in B cell growth phenotype reflect novel patterns of Epstein-Barr virus latent gene expression in Burkitt's lymphoma cells. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:2743–2751.
43. Gregory, C.D., C. Dive, S. Henderson, C.A. Smith, G.T. Williams, J. Gordon, and A.B. Rickinson. 1991. Activation of Epstein-Barr virus latent genes protects human B cells from death by apoptosis. *Nature (Lond.).* 349:612–614.
44. Gregory, C.D., T. Turz, C.F. Edwards, C. Tetaud, M. Talbot, B. Caillou, A.B. Rickinson, and M. Lipinski. 1987. Identification of a subset of normal B cells with a Burkitt's lymphoma (BL)-like phenotype. *J. Immunol.* 139:313–318.
45. Denepoux, S., D. Razanajaona, D. Blanchard, G. Meffre, J.D. Capra, J. Banchereau, and S. Lebecque. 1997. Induction of somatic mutation in a human B cell line in vitro. *Immunity.* 6:35–46.
46. Askew, D.S., R.A. Ashmun, B.C. Simmons, and J.L. Cleveland. 1991. Constitutive c-myc expression in an IL-3–dependent myeloid cell line suppresses cell cycle arrest and accelerates apoptosis. *Oncogene.* 6:1915–1922.
47. Evan, G.I., A.H. Wyllie, C.S. Gilbert, T.D. Littlewood, H. Land, M. Brooks, C.M. Waters, L.Z. Penn, and D.C. Hancock. 1992. Induction of apoptosis in fibroblasts by c-myc protein. *Cell.* 69:119–128.
48. Hermeking, H., and D. Eick. 1994. Mediation of c-myc–induced apoptosis by p53. *Science (Wash. DC).* 265:2091–2093.
49. Harrington, E.A., M.R. Bennet, A. Fanidi, and G.I. Evan. 1994. C-myc induced apoptosis in fibroblasts is inhibited by specific cytokines. *EMBO (Eur. Mol. Biol. Organ.) J.* 13: 3286–3295.

50. Goodnow, C.C., J. Crosbie, S. Adelstein, T.B. Lavoie, S.J. Smith-Gill, R.A. Brink, H. Pritchard-Briscoe, J.S. Wotherspoon, R.H. Loblay, K. Raphel, et al. 1988. Altered immunoglobulin expression and functional silencing of self-reactive B lymphocytes in transgenic mice. *Nature (Lond.).* 334:676–682.
51. Uchida, T., T. Matozaki, K. Matsuda, T. Susuki, S. Matozaki, U. Nakano, K. Wada, Y. Konda, C. Sakamoto, and M. Kasuga. 1993. Phorbol ester stimulates the activity of a protein tyrosine phosphatase containing SH2 domain (PTP1C) in HL-60 leukemia cells by increasing gene expression. *J. Biol. Chem.* 268:11845–11850.
52. Zhao, Z., S.-H. Shen, and E.H. Fischer. 1994. Phorbol ester–induced expression, phosphorylation, and translocation of protein-tyrosine-phosphatase 1C in HL-60 cells. *Proc. Natl. Acad. Sci. USA.* 91:5007–5011.
53. Galibert, L., N. Burdin, B. De Saint-Vis, P. Garrone, C. Van Kooten, J. Banchereau, and F. Rousset. 1996. CD40 and B cell antigen receptor dual triggering of resting B lymphocytes turns on a partial germinal center phenotype. *J. Exp. Med.* 183:77–85.