Differential Involvement of the Transcription Factor Blimp-1 in T Cell-Independent and -Dependent B Cell Differentiation to Plasma Cells


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Differential Involvement of the Transcription Factor Blimp-1 in T Cell-Independent and -Dependent B Cell Differentiation to Plasma Cells


Along humoral immune responses, different stimuli drive the differentiation of B lymphocytes to Ig-secreting plasma cells in discrete microenvironments. The Blimp-1 transcription factor is up-regulated early during the transition of mature B cells to IgM-secreting plasma cells. In the present study, we have examined the requirement of Blimp-1 in plasma cell formation after both T cell-independent (LPS) and -dependent (CD40 + IL-4, Th cell lines) stimulation of spleen B cells. B lymphocyte-induced maturation protein (Blimp-1) was expressed early after in vitro LPS stimulation, mainly in a population of IgM+/Syndecan−/CD43+ preplasma cells. In contrast, the BSAP transcription factor expressed in mature B cells was down-regulated during the differentiation to plasma cells. Treatment of these cultures with Blimp-1-specific antisense phosphorothioate oligonucleotides suppressed both Blimp-1 protein levels and the emergence of IgM+/Syndecan− cells and plasma cells. However, T-B cell cocultures of spleen B cells from C3H/HeJ (H-2b) mice and syngeneic autoreactive SR.10 Th2 cells submitted to the anti-Blimp-1 therapy did not show any significant reduction in IgM- and IgG1-secreting plasma cell formation. Spleen B cells treated with anti-CD40 mAb + IL-4 differentiated to IgG1-secreting cells without significant transcription of the Blimp-1 gene; anti-Blimp-1 treatment subsequently did not have any effect in the later cultures. Altogether, these results suggest that Blimp-1 transcription factor specifically promotes T cell-independent B cell differentiation to plasma cells, probably at preplasma cell stages. In contrast, T cell-dependent plasma cell formation likely evolves through Blimp-1-independent pathways. The Journal of Immunology, 1999, 163: 611–617.

The transcription factor, B lymphocyte-induced maturation protein (Blimp-1), has been postulated to act as a positive regulator of B cell maturation to Ig secretion (6). The human equivalent of Blimp-1, called PRDI-BF1, is a repressor of β-interferon gene expression and shares homologous domains with the retinoblastoma-binding RIZ protein, which was also implicated in cellular differentiation (7). Mouse Blimp-1 expression correlates with plasma cell generation in vitro established cell lines. The transfection of either transformed mature B cell lines or anti-IgM-arrested B cells with a Blimp-1 expression vector induced the appearance of IgM-secreting cells (6, 8). We decided to elucidate the requirement for Blimp-1 expression in both T cell-independent and -dependent pathways of physiological plasma cell formation, as well as to better define the developmental stages of its action. Our results revealed a very early induction of Blimp-1 expression after LPS polyclonal B cell stimulation, clearly preceding the differentiation to IgG1-secreting cells. Blimp-1 was first expressed in a population of blastic IgM+Syndecan-1(Synd)+CD43+ preplasma cells. Both the emergence of the later cell population and differentiation to IgM-secreting plasma cells were suppressed by Blimp-1-specific antisense oligonucleotides (ON). In contrast, plasma cell differentiation and IgM/IgG1 class switching secondary to Th cell stimuli (Th2 cell line, anti-CD40 + IL-4) were neither accompanied by Blimp-1 up-regulation nor affected by anti-Blimp-1 ON treatments.

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3 Abbreviations used in this paper: Blimp-1, B lymphocyte-induced maturation protein; Synd, Syndecan-1; ON, oligonucleotides; Aś, antisense; S, sense; NS, non-sense; ELISPOT, enzyme-linked immunospot; L, ligand.
ROLE OF BLIMP-1 IN T CELL-DEPENDENT PLASMA CELL FORMATION

Materials and Methods
Mice, cell cultures, and proliferation assays
BALB/c and C3H/HeJ mice, 8–12 wk old, were maintained in the specific pathogen-free animal facilities of the Instituto de Salud Carlos III. Total splenocytes and purified B cells (see below) were cultured at 10^6 cells/ml in RPMI 1640 medium (Life Technologies, Paisley, U.K.) supplemented with 10% FCS (Lantech, Uckfield, U.K.), 2 mM L-glutamine, 1 mM pyruvate, 50 mM 2-ME, 10 mM HEPES, and antibiotics. Cultures were maintained at 37°C and 5% CO2 in a humidified atmosphere.

Escherichia coli LPS (Difco, Detroit, MI) was added to 25 μg/ml. The SR.D10 cell line is an autoantigenic variant of the CD4+ Th2 cell line DIO14.G1.1, known to secrete IL-4, IL-6, and IL-10 (9, 10), and it was maintained in vitro by coculture with mitomycin C-treated, syngeneic C3H/HeJ feeder splenocytes in a 1:50 ratio (see below) (11). For Th cell cultures, SR.D10 cells were cocultured with purified spleen B cells (cell ratio of 1 SR.D10 vs 100 splenic B cells). Cells with anti-CD40 FGK45.5 mAb (10 μg/ml) and mouse rIL-4 (100 U/ml) were set up as described before (11). Cell proliferation was measured after a 12-h pulse of 1μCi[^H]thymidine, and incorporated radioactivity was quantified by scintillation counting (Wallac LKB, Turku, Finland).

Flow cytometry analysis and sorting
Fluoresceinated anti-leukiossianin 57 (anti-CD43) (12) and biotinylated anti-Synd-1 (13) mAbs were purchased from PharMingen (San Diego, CA). Anti-IgM (331.12) (14) mAb was purified from culture supernatants by affinity chromatography on protein G columns (Pharmacia, Uppsala, Sweden) and fluoresceinated by standard methods. Phycoerythrin-conjugated Streptavidin was from Southern Biotechnology Associates (Birmingham, AL). Two-color stainings were performed as previously described (15). Dead cells were excluded on the basis of forward- and side-light scatter parameters, and propidium iodide staining. Specific mAb signals were defined against the fluorescence provided by isotype-matched, irrelevant mAb. Flow cytometry analyses were performed on an EPICS-XL flow cytometer (Coulter Electronics, Hialeah, FL). Cell sorting of splenic IgM+ B cells and cultured IgM+Sind + cells were undertaken in a FACStar (Becton Dickinson, Mountain View, CA), and reanalyses of the purified cell samples were done in the EPICS-XL analyzer, to control the degree of purity. The frequencies of contaminating cells in purified samples were <5% after flow cytometry reanalysis.

ELISPOT assays
Ninety-six-well ELISA plates (Nunc, København, Denmark) were precoated with 10 μg/ml anti-IgM antibody. B cells from mice IgM−/− (Splenic, Mucosal, IgM−/−) mAb (Southern Biotechnology Associates) for detection of IgM- and IgG1-secreting cells, respectively (3 μg/ml), and blocked with 1% gelatin/ PBS. Cells were removed from cultures, washed three times, and counted. Serial diluted cells were seeded in triplicate in 1% FCS/RPMI 1640 and incubated at 37°C overnight in the coated plates. After washing, IgM- and IgG1-secreting cells were detected by incubation with biotinylated goat anti-mouse IgM (Southern Biotechnology Associates) or goat anti-mouse IgG1 mAb (187.1) (16), respectively. The plates were washed again and incubated with Streptavidin-conjugated alkaline phosphatase (Southern Biotechnology Associates). Plates were developed by adding 5-bromo-4-chloro-3-indolyl-phosphate (Sigma) in alkaline buffer containing 1% low melt agarose (Sigma) and incubated at 37°C. Developed spots were counted under a dissecting microscope.

RT-PCR analyses
Total RNA was isolated and cDNA was prepared as described (17), using 1 μg oligo(dT) as primer and avian myeloblastosis virus reverse transcriptase (Promega, Madison, WI). Equal amounts of cDNA were used to amplify Blimp-1, BSAP, and β-actin transcripts. Enzymatic amplifications were performed as follows: initial denaturation (95°C, 5 min), incubation (80°C, 5 min), addition of 2.5 U of Taq DNA polymerase (DNAzyme, Esperanza, Finland) and 30 cycles of the following steps: denaturation (95°C, 1 min), annealing (60°C, 1 min), and elongation (72°C, 1 min). The polymerization step was extended for 10 min more. Primers for β-actin are described (15); for Blimp-1 and BSAP amplifications, the following primers were designed: Blimp-1 sense; 5’-TCA GCCCTTCTCCATGTT TGATC-3’; Blimp-1 reverse; 5’-AATCTAAAGACTCCATCGT TCAAC-3’; BSAP sense; 5’-CAGCAGTCTGCTAAGGCT-3’; BSAP reverse; 5’-GTCTGGCGGCTGAAATAGG-3’. The amplification product (one-fifth of each) was separated electrophoretically on 1.5% agarose gels and then transferred to Zeta-probe membranes (Bio-Rad, Hercules, CA) after treatment with 0.4 M NaOH. Hybridization was performed with β-actin and Blimp-1 probes, 32P-labeled by random priming (18). The β-actin insert was the PstI fragment of the plasmid p41AL (19). The Blimp-1 insert was obtained after cloning the 178-bp fragment of the PCR-amplified Blimp-1 from LPS-activated B cells into the Bluescript vector (Stratagene, La Jolla, CA). The following internal ON was used as a 32P-labeled BSAP probe: 5’-AAGCCATGAGC GAACTACCTGTG-3’. PCR signal intensities were quantified by densitometry (Fujifilm-1000 detector, Fuji, Tokyo, Japan). Control experiments of the primers were performed in order to ensure the quantification of the cDNA samples: aliquots of the amplifications were removed at 20, 25, 30, or 35 cycles in preliminary PCRs, and serial dilutions of the positive cDNA samples were PCR amplified for 35 cycles.

In vitro protocols with phosphorothioate-modified ON
Phosphorothioate ON were prepared by Isogen Bioscience BV (Maarsen, The Netherlands). These consisted of three 18-mer that were antisense (AS), sense (S), and non-sense (NS) for the DNA sequence spanning the fragment of the murine Blimp-1 cDNA located −4 to +14 from the first ATG: AS: 5’-TAAGGCTTCTCCATGTTG-3’; S: 5’-GGACATGAGGAGGCTTA-3’; NS: 5’-AARSWRRWSRWRWRWWRW-3’ (W: A + T; R: A + G; S: G + C).

Spleen B cells were purified by cell sorting, washed twice with BSS, and then cultured in serum-free Opti-MEM (Life Technologies) supplemented with 5% NCTC 109 medium (BioWhittaker, Walkersville, MD) in basal conditions, under stimulation with LPS, anti-CD40 mAb + IL-4, or cocultured with SR.D10 cells, as described above. Different concentrations of ON were added at the start of cultures and for various lengths of time.

Western blot analysis
Cell extracts (10^6 cells/sample) were obtained after lysis in: 20 mM Tris-HCl (pH 7.5), 200 mM KCl, 10 mM EDTA, and 1 mM PMSF. The resulting samples were boiled for 5 min in 2× SDS Laemmli sample buffer (0.04 M Tris-HCl (pH 6.8), 2% SDS, 5% β-mercaptoethanol, 10% glycerol, 0.002% bromphenol blue), electrophoresed in SDS-PAGE (6%), and then transferred to Hybond ECL nitrocellulose membranes (Amersham Life Science, Little Chalfont, U.K.). The total amount of protein/sample was determined before loading, using a protein determination reaction (Bio-Rad). As a further loading control, the lower part of the gel was sliced and stained with Coomasie blue, and two selected bands/ lane were quantified (Gelworks 1-D intermediate program, UVP, Cambridge, U.K.). Filters were blocked with 5% fat-free dry milk in PBS/0.05% Tween-20 and incubated overnight with anti-Blimp-1 rabbit polyclonal antiserum (generously provided by Dr. M.M. Davis, see Ref. 6), washed, and further incubated with HRP-conjugated anti-rabbit Ig (Southern Biotechnology Associates; 2 h at room temperature). Filters were developed using ECL Western blotting detection reagent (Amersham).

Results
The Blimp-1 gene is selectively up-regulated in IgM+Sind “CD43+” preplasma cells
Although it is absent in pre-B/B cell lines, Blimp-1 expression is detected in tumors with plasma cell phenotype and in plasma cell-containing cultures (6). We have dissected the kinetics of Blimp-1 transcription in normal splenocytes activated with LPS mitogen. The appearance of plasma cell markers in flow cytometry, Ig-secretory cells in ELISPOT assays, and Blimp-1 transcripts in specific RT-PCR were analyzed in parallel. Blimp-1 was well expressed after a few hours of LPS culture, long before significant numbers of IgM-secreting cells were detected in the same cultures (24 to 28 h) (Fig. 1A, white triangles). Blimp-1 specific transcripts reached half of their maximum values (obtained in 72-h cell cultures) at 18–24 h, when only small numbers of IgM-plasma cells relative to those of IgM-secreting cells obtained at 72 h) had developed. The mean ratio between specific Blimp-1 gene expression and IgM plasma cells subsequently peaked at early time points (18 h) and later decreased, although IgM plasma cells were still expanding in the cultures (Fig. 1A, black circles). These data show that the Blimp-1 gene is up-regulated very early after LPS B cell stimulation, and probably at cellular stages previous to fully mature plasma cells.
They were performed on normal splenocytes, 72-h LPS spleen cell cultures, and 72-h LPS IgM frequencies of IgM shown), although it also included other preplasma cell stages. The creating cells of the cultures (as observed after its purification with analyzing the Blimp-1 transcripts present in IgM plasma cell transitional stage. We tested this possibility directly by 1 present in the same cultures. The early Blimp-1 transcription higher (10-fold) than the relative number of IgM-secreting cells BSAP not shown). In contrast, transcription of the cultured cells were detected in 72-h LPS cultures. The ratios between Blimp-1 transcription levels and IgM-secreting cells were calculated (right scale of ordinates). The data obtained in five independent experiments are shown in the figure (mean ± SD). B, Two-color stainings of LPS-stimulated BALB/c splenocytes were performed at the starting point and in 72-h cultures by using fluoresceinated anti-CD43 and anti-IgM mAb, and biotinylated anti-Synd mAb, as described in Materials and Methods. Cell size (as defined by forward scatter) is shown in histograms for normal splenocyte (left histogram), and for 72 h LPS IgM Synd and IgM Synd cell populations (right histograms: shaded and empty areas, respectively). All data are displayed in log\(_{10}\) fluorescence scales, except for forward light scatter (FSC), which is linear. In the bottom, RT-PCR assays for Blimp-1, BSAP, and Synd 1 genes were quantified by densitometric analyses. The Blimp-1 message levels were defined for each time point as follows: 100 × (OD Blimp-1/OD ß-actin)/(maximal OD of Blimp-1/maximal OD of ß-actin). A total of 107,000 ± 16,000 IgM-secreting cells/10\(^6\) cultured cells in the LPS cultures were 10 –20 times higher in the purified IgM Synd cells than in total LPS cultures (Fig. 1B). Blimp-1 transcripts were barely detected in the sorted Synd population of the same cultures (data not shown). In contrast, transcription of the BSAP gene, expressed in mature B cells (5, 21), was strongly down-regulated in the LPS cultures and in the purified IgM Synd cells (Fig. 1B).

Mitogenic stimulation of spleen B cells induced their entry into G1, an increase in cell size (as shown by large forward scatter in FACS; histograms of Fig. 1B) and progression through the rest of the cell cycle. A fraction of these activated B cells progressively acquired features of differentiating plasma cells. We analyzed in the LPS stimulated cultures the expression of CD43 and Synd-1 Ags, which are normally present in bone marrow pre-B cells, down-regulated in mature B cells, and reexpressed again in IgM-secreting plasma cells (12, 20). A novel population of blasts Synd\(^+\)CD43\(^+\) cells emerged very early in the cultures (2% in 24-h cultures) and expanded during the following days (5% and 10% at 48 and 72 h, respectively) (Fig. 1B, and data not shown). The IgM Synd cell population contained most of the IgM-secreting cells of the cultures (as observed after its purification with FACs sorting and IgM-specific ELISPOT analyses; data not shown), although it also included other preplasma cell stages. The frequencies of IgM Synd CD43\(^+\) cells in the LPS cultures were higher (10-fold) than the relative number of IgM-secreting cells present in the same cultures. The early Blimp-1 transcription shown above (Fig. 1A) suggested that this gene might be selectively up-regulated at the level of the IgM Synd CD43\(^+\) B cell plasma cell transitional stage. We tested this possibility directly by analyzing the Blimp-1 transcripts present in IgM Synd cells purified by FACs sorting, in quantitative RT-PCR analyses. The relative expression of Blimp-1 (compared with the one of ß-actin) was 10–20 times higher in the purified IgM Synd cells than in total LPS cultures (Fig. 1B). Blimp-1 transcripts were barely detected in the sorted Synd population of the same cultures (data not shown). In contrast, transcription of the BSAP gene, expressed in mature B cells (5, 21), was strongly down-regulated in the LPS cultures and in the purified IgM Synd cells (Fig. 1B).

Inhibition of Blimp-1 protein expression suppresses the generation of IgM Synd preplasma and IgM-secreting plasma cells in LPS-activated spleen B lymphocytes

Blimp-1 expression was previously shown to be sufficient for plasma cell maturation, as demonstrated by Blimp-1 transfection into either nonsecreting B cell lines or anti-IgM-blocked B cells (6, 8). We asked whether Blimp-1 activity was also necessarily required for plasma cell differentiation. Phosphorothioate-modified, Blimp-1-specific S, AS, and NS ON were generated and added to LPS-stimulated cultures of FACs-sorted, C3H/HeJ spleen B cells. To reduce sequence-independent effects of ON, we included no CpG motifs (B cell stimulatory) in their sequences, and we worked with low ON concentrations (1–10 \(\mu\)M) (22, 23). These experiments revealed a specific, dose-dependent reduction of the IgM Synd preplasma cell population in cultures treated with AS Blimp-1 ON (Fig. 2A). More important, the emergence of IgM-secreting plasma cells was progressively reduced in a time- and dose-dependent manner with the AS Blimp-1 therapy. Thus, IgM-secreting cells of AS Blimp-1 ON-treated cultures (3 \(\mu\)M) reached only one-third to one-fourth of those present in 3-day basal LPS cultures. Some nonspecific reductions in plasma cells were observed with both S and NS Blimp-1 ON, but they were significantly different from those induced by Blimp-1 AS ON (\(p < 0.05\), for all the ON doses in 72-h cell cultures, after applying the two-tailed unpaired Student \(t\) test) (Fig. 2B). Cell cultures extended until 96 h with 3 and 10 \(\mu\)M doses of ON provided similar results, although nonspecific cell death increased (data not shown). In order to check for the cellular level of action and the specificity of the anti-Blimp-1 ON treatment, we analyzed both Blimp-1 gene transcripts by RT-PCR and Blimp-1 protein levels by means of
Western blots with a Blimp-1 antiserum (6), in selected samples of the cell cultures. While Blimp-I gene transcription remained unmodified with/without ON treatments (Fig. 2C), the small Blimp-I protein levels detected in ex vivo B cells were augmented upon LPS stimulation (3-fold) and this increase was suppressed with AS ON, but not with S ON (Fig. 2D). Blimp-1 AS ON thus acted at the posttranscriptional level of expression. We here conclude that Blimp-I gene activity is necessary for normal spleen B lymphocyte differentiation to IgM-secreting plasma cells in T cell-independent conditions. The Blimp-1 role is first observed at the level of the generation of IgM+ Synd+ preplasma cells.

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LPS cultures, respectively; data not shown from three independent experiments). The findings described here suggest 1) that B cell proliferation rates are independent of Blimp-1 activity, and 2) that Blimp-1 may also be dispensable for T cell-driven plasma cell differentiation and Ig switching.

Anti-CD40 + IL-4 stimulation of spleen B cells gives rise to IgG1-secreting cells in the absence of Blimp-1

Anti-CD40 mAb and IL-4 cooperate in the differentiation of B cells to IgG1 plasma cells (25–27), and partially mimic conventional Th activities. Both stimuli together drive the B cells away from early IgM secretion and commit them to isotype switching and, thus, presumably to enter the long-lived plasma and memory cell pools (28). We set up cultures of spleen B cells stimulated with anti-CD40 mAb and IL-4 as a surrogate of T cell help (11), in the presence or not of Blimp-1 ON treatments. A population of IgG1-secreting cells newly appeared in the anti-CD40 + IL-4 cell cultures. Neither the IgM+Synd−CD43− cell subset nor a significant up-regulation of Blimp-1 gene transcription was observed, when compared with parallel LPS cell cultures (Fig. 4, A and B). While the numbers of IgG1-secreting cells did not change upon Blimp-1 ON therapy in anti-CD40 + IL-4 cell cultures, they were selectively decreased in LPS cell cultures submitted to the same Blimp-1 AS ON treatment (Fig. 4C). Cell cultures stimulated with either anti-CD40 mAb alone or with IL-4 alone did not show either the IgM+Synd−CD43+ cells, the Blimp-1 up-regulation, or IgG1-secreting plasma cells. A consistent decrease of basal Blimp-1 transcription levels was observed in the IL-4 enriched cell cultures, as has been described (29). BSAP transcripts of mature B cells were decreased in all the stimulated B cell cultures (IL-4, anti-CD40 mAb, anti-CD40 mAb + IL-4, and LPS), even in those cases in which Blimp-1 was not up-regulated (Fig. 4). We conclude that B cell differentiation to IgG1-secreting plasma cells secondary to CD40/CD40L + IL-4 signals follows a distinctive pathway in the absence of IgM+Synd−CD43+ preplasma cells, and without the requirement of Blimp-1 gene expression and activity.

Discussion

Expression of the zinc finger protein Blimp-1 correlates with the maturation of B lymphocytes to Ig-secreting plasma cells. Transfection experiments have also shown the Blimp-1 capacity to promote plasma cell differentiation (6, 8). Considering that Ig-secreting cells emerge at different time points of the humoral response and following various stimuli (28), we decided to study whether the Blimp-1 transcription factor was required by any pathway of plasma cell differentiation. In vitro treatment of activated B cells with AS ON specific to Blimp-1 revealed that 1) upon LPS stimulation, Blimp-1 was up-regulated in an early-appearing population of IgM+Synd−CD43+ preplasma cells; 2) Blimp-1 was necessary for the emergence of both preplasma and IgM-secreting cells in these cultures; 3) T cell-dependent B cell differentiation to IgG1-secreting cells was independent of Blimp-1 activity; and 4) Blimp-1 was not expressed in B cells stimulated by anti-CD40 + IL-4 during their evolution to IgG1-secreting cells. Altogether,
these findings suggest that, while Blimp-1 activity is required during the early (T cell-independent) plasma cell generation, it is dispensable to the late (T cell-dependent) emergence of Ig-switched plasma cells. Expression of the BSAP gene, involved in the positive regulation of B cell proliferation (5) and inhibition of IgA switching (21), is down-regulated in the B cell cultures, independently of Blimp-1 activity. Our findings on BSAP gene expression apparently disagree from others (5), which showed increases in BSAP protein levels on day 2 LPS- or CD40L-stimulated cultures. The discrepancy is likely due to the different kinetics of analyzed targets: BSAP transcription levels in 72-h cultures (this work) vs EMSA assays of nuclear BSAP protein activity (5).

B cell activation through the OX40/OX40L pathway, which is up-regulated by CD40 stimuli, also gave rise to Ig-secreting cells in the absence of Blimp-1 expression (30). Persistent stimulation of the CH12 B cell line with CD40L-transfected cell lines induced a down-regulation of Blimp-1 together with an arrest of B lymphocyte terminal differentiation (29). These later data do not disagree with ours because in physiological conditions activated T cells rapidly down-regulate CD40L (31, 32). Also, T cell-derived IL-4 concurs with CD40 in B cell differentiation to IgG1 plasma cells (26), while being unable to up-regulate Blimp-1 transcription (8, 29). The expression of Synd-1, normally controlled by Blimp-1 (6), was also shown to be absent during Th2-driven differentiation to IgG1+ cells (33). It has been described that cytokines can modulate Blimp-1 transcription (8). In particular, IL-6 is a major inducer of plasma cell differentiation (34) and up-regulates Blimp-1 transcription in the CH12 cell line (29). In recent experiments, we found small IL-6 transcript levels in LPS-stimulated B cells (not shown), as described for CH12 cells (34). The addition of IL-6 to the B cell cultures only provoked modest (3-fold) increases in Blimp-1 transcription. Moreover, LPS-activated B cells from IL-6-/- and wild-type mice (35) showed similar Blimp-1 transcription (our manuscript in preparation). Although we cannot completely exclude that IL-6 (and/or other cytokines) may contribute to the up-regulation of Blimp-1 by LPS, IL-6 is not a necessary requirement.

Other transcription factors are important at different levels of B cell activation/proliferation. Oct-2 and the OCA-B coactivator act on early B cell activation (36, 37). The different complexes of the NF-κB/Rel family of transcription factors play relevant roles during B cell proliferation and Ig switching (3). BSAP and c-myc regulate the growth rates of mature B cells (5), the later one being suppressed by Blimp-1 (38). c-myc down-regulation may be responsible for the apoptosis-inducing activity of Blimp-1 in selected cell lines (39). Our data show that BSAP down-regulation during T cell-dependent plasma cell differentiation (40) is not mediated by Blimp-1 activity.

Based on the above findings, it is tempting to speculate that distinctive molecular requirements are involved in the production of different types of plasma cells. A provisional scenario of plasma cell differentiation could be delineated (Fig. 5): Ag stimulation of naive B lymphocytes drives them to activation, proliferation (c-myc and BSAP dependent), clonal expansion, and a Blimp-1-controlled checkpoint of apoptosis/differentiation to plasma cells (39). The later selective step may occur at the level of IgM Synd CD43+ preplasma cells. Surviving cells will give rise to the early wave of IgM-secreting plasma cells of the primary response. T cell dependent, Ag-primed B cells receive signals for proliferation/survival from activated T cells (CD40L expressing) (41, 42), which bias them away from early death/plasma cell differentiation toward Ig-switched plasma cells (28). The expression of BSAP and the absence of Blimp-1 transcription factors (40) might be, among others, molecular characteristics of the T cell-dependent pathway of plasma cell differentiation.

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