Blimp-1 directly represses \(\text{Il2}\) and the \(\text{Il2}\) activator \(\text{Fos}\), attenuating T cell proliferation and survival

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The terminal differentiation of effector lymphocytes after encounter with antigen is a complex and tightly regulated process that ensures rapid, but limited, immune responses. Transcription factors play crucial regulatory roles in the differentiation processes that culminate in the formation of effector and memory B and T cells (1, 2). The transcriptional repressor B lymphocyte–induced maturation protein-1 (Blimp-1) is one of the few transcription factors known to be crucial for regulating B lymphocyte terminal differentiation. Blimp-1 is required (3) and sufficient (4) for the formation of fully functional antibody-secreting plasma cells and for maintenance of long-lived plasma cells in the bone marrow (5).

Blimp-1 is a SET domain and a zinc finger–containing transcriptional repressor encoded by the \(\text{Prdm1}\) gene. Transcriptional repression by Blimp-1 is mediated by repressive modifications in chromatin structure, through recruitment of Groucho family transcriptional corepressors, and chromatin-modifying enzymes (for review see [6]). In addition to its crucial role in the differentiation of plasma cells, Blimp-1 has critical functions in embryonic development (7, 8), and targeted deletion of the \(\text{Prdm1}\) gene in the mouse is embryonically lethal (9). Blimp-1 is also required for terminal differentiation of several nonlymphoid cell lineages in adult organisms (for review see [6]). Recently, a role for Blimp-1 in T cell differentiation was demonstrated in two laboratories (10, 11). After T cell receptor (TCR) stimulation, both CD4+ and CD8+ T lymphocytes express Blimp-1 mRNA in amounts comparable to that in fully mature plasma cells. Blimp-1 mRNA is also expressed at high levels in Foxp3+ CD4+ regulatory T cells. Conditional deletion of Blimp-1 in T cells results in profound alterations of T cell homeostasis and function and culminates in the spontaneous development of fatal colitis (10, 11). Blimp-1–deficient CD4+ regulatory T cells (T reg cells) are partially dysfunctional, as they perform properly in in vitro immunosupression assays and in one colitis model in vivo (11), but fail to inhibit chemically induced colitis in WT mice (10).

TCR stimulation of Blimp-1–deficient CD4+ T cells in vitro results in hyperresponsiveness.
that is revealed by robust proliferation in suboptimal stimulatory conditions and increased numbers of IL-2 producers, indicating that Blimp-1 is a negative regulator of IL-2 production (10). This idea is also reinforced by two recent observations: (a) Blimp-1 and IL-2 expression upon TCR stimulation are inversely correlated, and (b) enforced expression of Blimp-1 in T cells represses IL-2 production upon TCR stimulation (12, 13).

IL-2 was initially identified as an autocrine cytokine necessary for in vitro expansion of T cells (14). Subsequent studies indicated a role for IL-2 in promoting T cell expansion, survival, effector differentiation, and memory cell survival via promotion of IL-7R expression (15). Indeed, the stimulatory properties of IL-2 make it a therapeutic target, especially in AIDS and cancer, where IL-2 administration promotes T cell expansion in vivo (15, 16). IL-2 has also been shown to participate in the contraction of inflammatory responses, by programming activated CD4+ T cells for apoptosis (17, 18) and promoting the growth and survival of the innate CD4+FoxP3+T reg cells (15). Because of these effects, IL-2− or IL-2R−deficient mice exhibit a multifaceted autoimmune phenotype characterized by multiorgan inflammation, absence of T reg cells, and accumulation of autoreactive T cells (for review see [15]).

Interestingly, IL-2 represses its own expression in a classical negative-feedback loop that functions in a STAT-5−dependent manner (19). This finding, together with the observation that IL-2 is a potent inducer of Blimp-1 expression in T cells (12), led to the suggestion that Blimp-1 plays important roles in IL-2 autoregulation.

We have investigated this hypothesis, and report that Blimp-1 directly represses the Il2 gene and indirectly represses Il2 by repressing Fos, which encodes Fos; Fos is a component of AP-1, a strong activator of Il2 (Fig. 1 C, top left). As a consequence of the increased production of IL-2 in the absence of Blimp-1, CD4+ T cell proliferate more upon antigen-specific stimulation and are more resistant to cytokine deprivation–induced cell death. Attenuation of IL-2 production by Blimp-1 plays a role in an antigen-specific response in vivo. Thus, one important function of Blimp-1 in T cells is to attenuate IL-2 production upon antigen stimulation, by both direct and indirect gene repression.

RESULTS AND DISCUSSION
Blimp-1 attenuates IL-2 expression in the primary response

We have previously reported that lack of Blimp-1 results in increased proliferation and IL-2 production after polyclonal TCR stimulation (10). To further understand the mechanisms by which Blimp-1 regulates IL-2 production, we evaluated the kinetics of IL-2 production in an antigen-specific context. Blimp-1 conditional KO (CKO) mice were bred to OT2 TCR transgenic mice to generate antigen-specific, Blimp-1−sufficient (Ctrl) and −deficient (CKO) CD4+ T cells. When naive (CD44low) OT2 CD4+ cells were stimulated in vitro with APCs and cognate antigen, the percentage of IL-2−producing cells was significantly higher in the CKO than in the Ctrl cultures in all time points evaluated. In agreement with results from polyclonal stimulation (10), TCR restimulation 3 d after primary activation also resulted in more IL-2−producing cells in the CKO cultures (Fig. 1 A). CKO cells proliferated more robustly, especially when antigen was provided in lower doses (Fig. S1 A, available at http://www.jem.org/cgi/content/full/jem.20080526/DC1). Thus, Blimp-1 attenuates proliferation and the number of IL-2−producing cells upon antigen-specific stimulation, and attenuation of IL-2 production by Blimp-1 can be observed at the time of primary stimulation.

IL-2 is known to induce Blimp-1 in B cells (4), and most of the induction of Blimp-1 mRNA and protein upon T cell activation has been shown to depend on IL-2 production (12). Consistent with our observation of increased IL-2 production in Blimp-1 CKO T cells (Fig. 1 A) (10), forced expression of Blimp-1 in T cells decreases expression of IL-2 (12, 13), and IL-2 causes its own down-regulation (12, 19). Given these complicated interactions, we investigated the kinetics of Blimp-1 and IL-2 expression in normal T cells, measuring steady-state mRNA levels. At days 1 and 2 after stimulation of naive CD4+ T cells in vitro, IL-2 steady-state mRNA is more strongly induced than Blimp-1 mRNA. However, by day 3, Blimp-1 mRNA increases significantly and IL-2 mRNA decreases (Fig. 1 B). To study this at the single-cell level, we used a mouse in which Blimp-1 mRNA expression is reported by EGFP (7). Total spleen cells were stimulated in vitro and analyzed for IL-2 and GFP expression. 1 d after stimulation, <10% of the CD4+ T cells expressed GFP, whereas ~5% of cells expressed IL-2. 3 d after stimulation, GFP expression increased to 17%, whereas IL-2 expression decreased to <3% (Fig. 1 C, top right). Restimulation on day 3 resulted in increased expression of both IL-2 protein and Blimp-1 mRNA, and addition of IL-2 amplified Blimp-1 expression, while decreasing IL-2 production (Fig. 1 C, bottom). Strikingly, in all time points, very few cells were double-positive for GFP and Blimp-1.

Previous studies show that IL-2 production is tightly regulated (20, 21), and that even under optimal conditions not all T cells in a population will acquire the competence to transcribe the Il2 gene and synthesize IL-2 upon primary stimulation (22). The negative correlation between Blimp-1 expression and IL-2 production at the single-cell level (Fig. 1, B and C), along with the observation that more cells make IL-2 when Blimp-1 is absent (Fig. 1 A), provide evidence that expression of Blimp-1 is important in the exclusion of IL-2 production upon TCR stimulation. These results confirm and expand previous data (10, 12), supporting the model that upon T cell activation, most induction of Blimp-1 occurs secondary to IL-2 production and that the induced Blimp-1 participates in a regulatory loop to repress IL-2 expression.

Blimp-1 is required for cytokine deprivation–induced cell death after activation

IL-2 also has important effects on T cell apoptosis (18). After T cell activation, IL-2 production decreases, triggering passive cell death and limiting T cell numbers at the conclusion
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The presence of Blimp-1 was responsible for the increased survival of effector cells in this assay (Fig. 2). Thus, Blimp-1–dependent repression of IL-2 is important for passive cell death at the conclusion of an immune response.

Lack of Blimp-1 results in increased IL-2 and Fos mRNA

We wished to understand the molecular mechanism by which Blimp-1 controls IL-2 production. Although the production of IL-2 is regulated transcriptionally and posttranscriptionally (20, 21) because Blimp-1 is a transcriptional repressor, it probably regulates the transcription of the Il2 gene, either directly and/or indirectly. To test this hypothesis, we first analyzed steady-state levels of IL-2 mRNA in cells from Ctrl and CKO mice. Naive CD4+ cells from CKO mice had significantly higher levels of IL-2 mRNA compared with Ctrl cells. Addition of IL-2 at the time of replating abrogated the differences between Ctrl and CKO cells, indicating that the increased IL-2 production in the absence of Blimp-1 was responsible for the increased survival of effector cells in this assay (Fig. 2). Thus, Blimp-1–dependent repression of IL-2 is important for passive cell death at the conclusion of an immune response.
higher amounts of steady-state IL-2 mRNA than cells from Ctrl mice, before and at later time points after stimulation (days 2 and 3), but not earlier (Fig. 3 A). This suggests that low levels of Blimp-1 in naive cells (10) are sufficient to repress IL2 before activation. Alternatively, or in addition, the repression of IL2 by Blimp-1 in naive cells could be facilitated by the regulatory pathways operating before TCR stimulation, which differ considerably from the ones in place after stimulation (21). Upon activation of IL2 by NFAT, members of AP-1 family and NF-kB (21), the relatively low amounts of Blimp-1 are apparently overcome, and Blimp-1 cannot repress IL2. However, as Blimp-1 levels rise, Blimp-1 is once again able to repress IL2 transcription. If higher levels of Blimp-1 are, indeed, required to contain IL2 transcription upon TCR stimulation, it would also explain why restimulation at day 3 results in more IL2 mRNA in Blimp-1 CKO cells, as in these circumstances Blimp-1 expression in WT cells is further elevated (Fig. 1C; unpublished data). Repression of IL2 by Blimp-1 is also consistent with the recent observation that enforced expression of Blimp-1 represses IL-2 production upon TCR stimulation (12, 13).

Fos, a component of the AP-1 family of transcription factors, is one of the well-known transcriptional activators of the IL2 gene in T cells (23, 24). Because our previous studies (25) showed that Fos is a direct target of Blimp-1–dependent repression in keratinocytes, we asked if Blimp-1 repressed Fos in T cells. Blimp-1–deficient CD4+ T cells stimulated (as described in Materials and methods) showed increased levels of Fos steady-state mRNA (Fig. 3 B). Thus, Blimp-1 normally down-regulates the steady-state mRNA of both IL2 and its activator Fos.

**Blimp-1 directly represses the IL2 and Fos steady-state mRNAs**

Preliminary chromatin immunoprecipitation (ChIP) experiments showed increased specific binding of Pol II to the transcription initiation site of the IL2 gene in activated CKO CD4+ T cells compared with Ctrl cells (unpublished data). Because a direct correlation between Pol II binding and transcription has been previously demonstrated for the IL2 gene (26), this suggested that Blimp-1 directly represses IL2 transcription. To test this hypothesis and identify Blimp-1 response elements, we searched the genomic sequence (plus 10 kb upstream and 10 kb downstream of the transcriptional start site [TSS]) of the mouse and human IL2 genes, looking for Blimp-1 consensus binding sites (CBSs). We found 20 putative CBSs for Blimp-1 in the mouse IL2 gene (Table S1, available at http://www.jem.org/cgi/content/full/jem.20080526/DC1), and 8 of these sites (−7,252, −5,547, −4,795, −4,765, −4,221, −4,207, −3,982, and −1,861) were contained in an 8.4-kb region upstream of the IL2 TSS, which has been shown to be repressed by Blimp-1 in an in vitro reporter assay (12). We focused our efforts on these sites first; however, some sites were embedded in simple-sequence repeats and we were unable to design primers for efficient amplification of these regions. Thus, sites −3,982, −1,861, in the upstream region of the gene, and +10,215 and a region containing no Blimp-1 consensus sequence (+1,556 to +1,713 bp) relative to the TSS were investigated. We assayed binding of endogenous Blimp-1 by ChIP in WT CD4+ T cells after 6-d stimulation and restimulation in vitro, a condition where Blimp-1 expression is high (6). Out of the 3 Blimp-1 CBSs investigated, only site −1,861 was enriched for Blimp-1 binding (Fig. 4A). Sites −3,982 and +10,215, as well as the irrelevant site, showed no significant enrichment (Fig. 4A). Thus, Blimp-1 binds specifically at the site −1,861 of the IL2 gene.

Previous studies showed that an 8.4-kb region of the IL2 promoter, containing this site, conferred proper expression in...
the effects of Fos in regulating proliferation can only be observed when both FosB and Fos are missing (for review see [30]).

Lack of Blimp-1 leads to increased IL-2 production in vivo

Our data (Figs. 1–2 and 4), and that of others (12, 19), show that the transient nature of IL-2 production after T cell activation depends on induction of Blimp-1 by IL-2 and subsequent Blimp-1-dependent repression of IL-2 production via repression of Il2 and Fos. To explore the role of Blimp-1-dependent repression of IL-2 in vivo, we transferred Ctrl and CKO CFSE-labeled naive CD4+ OT2 CD4+ T cells were injected i.v. into CD45.1 congenic WT mice. 2 d later, mice were immunized i.v. with antigen-pulsed APCs, and 5 d later, lymph nodes were harvested and proliferation (A) and IL-2 production (B) were analyzed. Data in B are the mean (± SEM) of the percentage of IL-2-producing cells in the CD45.2+ gate in four different experiments. (C) Schematic representation of Blimp-1 participation in the IL-2 autoregulation. Induction/activation of N-FAT, AP-1, and NF-κB upon TCR and CD28 stimulation leads to IL-2 transcription. IL-2 acting via STAT-5 leads to Blimp-1 expression. Blimp-1 then represses the Il2 and Fos genes, inhibiting IL-2 production.

transgenic cells (27) and forced expression of Blimp-1 decreased expression of this transgene (12). Although another potential Blimp-1 CBS is present in the 8.4-kb region (site -3,982), this site does not seem to bind Blimp-1 in vivo (Fig. 5A). Thus, the −1,862-bp site is likely to mediate repression of Il2 by Blimp-1, although other currently untested sites in the 8.4-kb regulatory region (Table S1) may also contribute.

We also evaluated Blimp-1 binding to previously identified sites in Fos (25). Sites located at −179 and −4,990 bp from the TSS of mouse Fos are enriched for Blimp-1 binding in CD4+ T lymphocytes. These results, together with previous results (Figs. 1–3), demonstrate that Blimp-1 directly represses transcription of Il2 and the Il2 activator Fos.

We conclude that Blimp-1 attenuates IL-2 production both directly, by repression of the Il2 gene, and indirectly, by repression of Fos. Nevertheless, the contribution of Fos to the overall increase in Il2 transcription in the CKO cells is difficult to determine. Enforced expression of Fos in T cells caused elevated IL-2 production (28); however, deletion of Fos alone is insufficient to decrease IL-2 production (29), and the effects of Fos in regulating proliferation can only be observed when both FosB and Fos are missing (for review see [30]).

Lack of Blimp-1 leads to increased IL-2 production in vivo

Our data (Figs. 1–2 and 4), and that of others (12, 19), show that the transient nature of IL-2 production after T cell activation depends on induction of Blimp-1 by IL-2 and subsequent Blimp-1-dependent repression of IL-2 production via repression of Il2 and Fos. To explore the role of Blimp-1-dependent repression of IL-2 in vivo, we transferred Ctrl and CKO CFSE-labeled naive CD4+ OT2 (CD45.2) into allotype congenic recipients (C57BL/6 SJ CD45.1), and then immunized the recipients with dendritic cells pulsed with OVA peptide. 5 d later, cells were recovered from lymph nodes of recipients and restimulated in vitro, and IL-2 production was evaluated in the CD45.2+ gate in four different experiments. (C) Schematic representation of Blimp-1 participation in the IL-2 autoregulation. Induction/activation of N-FAT, AP-1, and NF-κB upon TCR and CD28 stimulation leads to IL-2 transcription. IL-2 acting via STAT-5 leads to Blimp-1 expression. Blimp-1 then represses the Il2 and Fos genes, inhibiting IL-2 production.
Thus, this study demonstrates that Blimp-1 represses IL-2 production after T cell activation and shows that the molecular mechanism responsible depends, at least in part, on Blimp-1-dependent repression of Il2 and Fos transcription. Furthermore, we identify Blimp-1 response elements in these two genes. The conclusion that Blimp-1 represses IL-2 transcription is supported by several observations: (a) Blimp-1–expressing cells do not express IL-2 protein at detectable levels; (b) Blimp-1 mRNA induction correlates with IL-2 mRNA downregulation; (c) IL-2 protein and steady-state mRNA are elevated in Blimp-1–deficient CD4+ T cells; and (d) endogenous Blimp-1 specifically binds to a regulatory region in the If2 gene in activated primary CD4+ T cells.

This establishes Blimp-1 as an important component in a recently described IL-2 autoregulatory loop (19) that operates in vivo to control the development and magnitude of T cell effector responses, and it confirms and extends the suggestion of Gong and Malek, that Blimp-1 plays an important role in IL-2 autoregulation (12).

Interestingly, after activation in vitro, IL-2 production in Blimp-1–deficient T cells eventually decreases, although more slowly than in Blimp-1–sufficient cells (Fig. 1 and Fig. 3 A). Thus, mechanisms in addition to Blimp-1 are apparently present for IL-2 downregulation. Nonetheless, Blimp-1 is important physiologically for IL-2 repression because lack of Blimp-1 results in increased IL-2 production upon antigen immunization in vivo. Increased levels of IL-2 produced by KO cells are associated with increased proliferation both in vitro and in vivo (Fig. S1 and Fig. S5), and with increased resistance to cytokine deprivation–induced cell death after activation in vitro (Fig. 2). Thus, deregulation of IL-2 production in the Blimp-1 CKO mice is likely to contribute to the aberrant cell homeostasis and the inflammatory phenotype observed in these mice.

**MATERIALS AND METHODS**

**Mice.** C57BL/6 (B6) and B6 CD45.1 congenic mice were purchased from The Jackson Laboratory. *Pdm1fl ox/fl ox* (3) were backcrossed 10 times with B6 mice, and then crossed with B6CD4-Cre mice, which were purchased from Taconic, to generate B6 *Pdm1fl ox/fl ox;CD4-Cre* (Blimp-1CKO) and *Pdm1fl ox/fl ox;Lck-Cre Pdm1fl ox/fl ox;Lck-Cre* (Control). For some in vitro experiments, mixed B6 × 129 *Pdm1fl ox/fl ox;Lck-Cre Pdm1fl ox/fl ox;Lck-Cre* (10) were also used. Mice bearing a BAC transgene–encoding membrane-target EGFP (mEGFP) under the control of Blimp-1 regulatory elements (Blimp-1 EGFP) (7) were a gift from M. Nussenzweig (The Rockefeller University, New York, NY) and were used as a reporter of Blimp-1 mRNA expression. In these mice, EGFP expression closely recapitulates Blimp-1 mRNA expression (7). For some experiments, spleen cells from Blimp-1–reporter mice were provided by S. Kaech’s laboratory (Yale University, New Haven, CT). All mice were maintained in a specific pathogen–free animal facility at Columbia University and handled in accordance with the institutional guidelines. Animal experiments were approved by the Institutional Animal Care and Use Committee at Columbia University.

**Cell isolation, stimulation, and IL-2 production.** Naive CD4+ (CD44lo) cells were sorted using a FACSAria fluorescent cell sorter (BD Biosciences). Routinely, purity of all cell preparation was >90%. For Blimp-1 ChIP experiments, naive CD4+ T cells were purified by negative selection using FITC–labeled antibodies and αFITC magnetic beads. The negatively isolated fraction contained >90% CD4+ cells. Naive B6 OT2-TG *Pdm1fl ox/fl ox* CD4–Cre or *Pdm1fl ox/fl ox;CD4-Cre* cells were stimulated in 48-well plates at a 1:1 ratio with APC (T cell–depleted and mytomycin–treated spleen cells) in a total of 5 × 10^5 cells per well with chicken OVA peptide (OVA 323–339; BPL0-910 H2N-ISQAVHAAHAEINEAGR-OH; New England Peptide) for 3 d. IL-2 production was determined after incubation with 10 μg/ml Brefeldin A (BFA; Sigma-Aldrich) for the last 2 h of culture. When indicated, restimulation was done with 50 ng/ml PMA and 500 ng/ml ionomycin (both from Sigma-Aldrich) for 4 h, with BFA added in the last 2 h. IL-2 was detected by intracellular staining in combination with surface CD4 (both antibodies from BD Biosciences). Cells were analyzed in a LSRII flow cytometer (BD Biosciences). IL-2 staining was evaluated exclusively in the live, CD4+ gated lymphocytes.

Simultaneous detection of Blimp-1 expression and IL-2 production. To evaluate IL-2 expression in Blimp-1–EGFP reporter cells, total spleen cells were stimulated with 1 μg/ml soluble αCD3 and 0.5 μg/ml αCD28 (both from BioExpress) with or without HU-I2 (QIAGEN). GFP expression was detected in permeabilized cells using an αGFP antibody (rabbit αGFP; Rockland Immunochemicals, Inc.) added simultaneously with the αCD-2 antibody. The αGFP antibody specifically stained GFP produced under the stimulation of the Blimp-1 transgenic promoter, as no staining was detected in nontransgenic cells stimulated and stained in the same way (Fig. 1 C).

**Quantitative real-time PCR.** For detection of Blimp-1, IL-2, and Fos mRNAs, naive CD4+ T cells were stimulated with 5 μg/ml plate-bound αCD3, 2.5 μg/ml μ/μ/ml αCD28, and 25 U/ml IL-2 for various time points. Total mRNA was isolated using TRIzol reagent (Life Technologies) according to the manufacturer’s instructions. Reverse transcription was performed on equal amounts of RNA for each sample using SuperScript III (Invitrogen). SYBR Green incorporation quantitative real-time PCR was performed using a FastStart SYBR Green mix (Roche) in the ABI7400 Sequence Detection System (Applied Biosystems). Primers used were as follows: IL-2 forward, 5'-AGACGCTGTIGATGACGCACTA-3'; IL-2 reverse, 5'-CGCAAGGTCCAGTTTCA-3' (designed using Primer 3 software). Primers for Blimp-1 and Fos were previously described (10, 25).

**ChIP.** ChIP assays were performed as previously described (25), with few modifications. Cells were fixed with 1% paraformaldehyde for 10 min at room temperature. Sonicated chromatin from 4–5 × 10^7 cells was immunoprecipitated with 25 μl of either rabbit αBlimp-1 polyclonal antibody serum (clone 267) or preimmune serum as a control. SYBR Green incorporation quantitative real-time PCR was performed in DNA recovered from immunoprecipitation and input samples (primers sequences in Table S2, available at http://www.jem.org/cgi/content/full/jem.20080526/DC1). Fold enrichment was calculated dividing the percentage of input values obtained with αBlimp-1 by the one obtained with Ctrl antibody. Analysis of sequence homology and identification of putative Blimp-1 consensus sites were performed using the ECR browser (http://ecrbrowser.dcode.org) and RNA 2.0 software. Genomic sequences were obtained from Ensembl.

**In vivo immunization.** CFSE–labeled naive B6 OT2-TG *Pdm1fl ox/fl ox* CD4–Cre or *Pdm1fl ox/fl ox;CD4-Cre* cells (CD45-2+) (1 × 10^6) were transferred (i.v.) to adoptively marked congenic recipients (C57Bl/6 SJ CD45.1). 2 d after cell transfer, mice were immunized i.v. with GMCSF–bone marrow–derived dendritic cells matured with LPS and IL-4 and pulsed with the cognate peptide (1 μg/ml OVA 334–339). 5 d later, lymph nodes were recovered and CFSE dilution was measured in the CD45-2+ cells. Alternatively, freshly harvested total lymph node cell suspensions were restimulated in vitro with PMA and ionomycin (with BFA added in the last 2 h), and IL-2 production was evaluated at the single–cell level in the CD45-2+ population by intracellular cytokine staining.

**Cytokine deprivation cell death assay.** Naive CD4+ T cells were stimulated (as described in Quantitative real-time PCR) for RNA isolation and
cultured for 5 d. On day 5, cells were washed extensively in complete RPMI medium and replated at the same initial density in the presence (50 U/ml) or absence of rHuIL-2. The percentage of live cells (7-AAD−/Annexin V−) was assayed 1, 2, or 3 d later.

**Online supplemental material.** Fig. S1 shows that lack of Blimp-1 results in increased proliferation upon antigen-specific stimulation in vitro. Table S1 shows the putative Blimp-1 binding sites at the I2 gene. Table S2 lists the primers used for Blimp-1 ChIP at the I2 gene. The online version of this article is available at http://www.jem.org/cgi/content/full/jem.20080526/DC1.

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