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Endoplasmic Reticulum Stress Regulator XBP-1 Contributes to Effector CD8+ T Cell Differentiation during Acute Infection

Daisuke Kamimura and Michael J. Bevan

The transcription factor X-box-binding protein-1 (XBP-1) plays an essential role in activating the unfolded protein response in the endoplasmic reticulum (ER). Transcribed XBP-1 mRNA is converted to its active form by unconventional cytoplasmic splicing mediated by inositol-requiring enzyme-1 (IRE-1) upon ER stress. We report activation of the IRE-1/XBP-1 pathway in effector CD8+ T cells during the response to acute infection. Transcription of unspliced XBP-1 mRNA is up-regulated by IL-2 signals, while its splicing is induced after TCR ligation. Splicing of XBP-1 mRNA was evident during the expansion of Ag-specific CD8+ T cells in response to viral or bacterial infection. An XBP-1 splicing reporter revealed that splicing activity was enriched in terminal effector cells expressing high levels of killer cell lectin-like receptor G1 (KLRG1). Overexpression of the spliced form of XBP-1 in CD8+ T cells enhanced KLRG1 expression during infection, whereas XBP-1 knockout (XBP-1−/−) CD8+ T cells or cells expressing a dominant-negative form of XBP-1 showed a decreased proportion of KLRG1high effector cells. These results suggest that, in the response to pathogen, activation of ER stress sensors and XBP-1 splicing contribute to the differentiation of end-stage effector CD8+ T cells. The Journal of Immunology, 2008, 181: 5433–5441.

The CD8+ T cell response to acute infection entails vigorous expansion of Ag-specific cells, generating effector cells that are able to secrete various cytokines and cytotoxic molecules. Following acute infection, naive Ag-specific CD8+ T cells may divide more than 14 times within 1 wk and acquire effector functions that contribute to eradication of the pathogen (1). Although most effector cells undergo programmed cell death when the infection is controlled, a small number are left behind and maintained as long-lived memory cells (2). Although the mechanisms underlying this cell fate decision are still debated, cell surface markers distinguishing these subsets have been identified. Following down-regulation of CD127 expression on activated T cells, a number of groups have reported that IL-2 plays a critical role in CD8+ T cell responses to infection. For example, IL-2 injection during viral infection differentially affects CD8+ T cell responses, depending on the timing of the treatment (6). In addition, CD8+ T cells lacking CD25, a component of the high-affinity IL-2R, expand severalfold less and form a greater proportion of CD62Lhigh central memory phenotype cells than do wild-type (WT) cells (7–9). In addition, CD25−/− memory CD8+ T cells are normally maintained, but are incapable of robust recall responses (8, 9). Treatment of mice with IL-2/anti-IL-2 mAb complexes (IL-2 complex) is a useful way to deliver IL-2 signals in vivo (10, 11). Following IL-2 complex treatment, naive CD8+ T cells differentiate into effector and memory-like cells without exogenous Ag stimulation (12). These observations indicate a diverse regulatory role for IL-2 signals, driving cells to full effector and memory differentiation, and led us to look for IL-2 target genes that affect CD8+ T cell responses.

ER stress caused by the accumulation of unfolded proteins in the ER activates the unfolded protein response (UPR), a mechanism that restores homeostasis in the ER. This response includes up-regulation of ER chaperones and of proteins involved in ER-associated protein degradation. Many factors are known to cause ER stress, including massive synthesis of secretory proteins, changes in calcium concentration, imbalance of the redox state, and nutrient deprivation (13–15). There are three major sensors of ER stress that trigger UPR, namely the pathways using the factors inositol-requiring enzyme-1 (IRE-1) and X-box-binding protein-1 (XBP-1) (IRE-1/XBP-1), activating transcription factor (ATF)6, and protein kinase R-like ER kinase (PERK) and eukaryotic translation initiation factor 2alpha (eIF2α) (PERK/eIF2α) (13–15). XBP-1 is a basic leucine zipper family transcription factor, the activity of which is uniquely regulated by an unconventional cytoplasmic splicing reaction mediated by IRE-1, an ER resident kinase/RNase. XBP-1 mRNA is synthesized as a precursor form with an extra intron of 26 nucleotides (16, 17). This unspliced form of mRNA (XBP-1u) has an open reading frame containing a DNA binding

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3 Abbreviations used in this paper: KLRG1, killer cell lectin-like receptor G1; ER, endoplasmic reticulum; ETAE, ER stress-activated indicator; ATF, activating transcription factor; eIF2α, eukaryotic translation initiation factor 2α; IRE-1, inositol-requiring enzyme-1; XBP-1, X-box-binding protein-1; LCMV, lymphocytic choriomeningitis virus; PERK, protein kinase R-like ER kinase; p.i., postinfection; UPR, unfolded protein response; WT, wild type.

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domain in the N terminus, and a degradation motif and nuclear exclusion signal in the C terminus (18). Upon ER stress, IRE-1 is activated and splices out the 26-nt intron, causing a frame shift of the coding region of XBP-1. The resulting spliced form (XBP-1s) encodes a longer polypeptide that retains the DNA binding domain, with a newly generated transactivating domain in the C terminus (16, 17). Microarray analyses using XBP1−/− embryonic fibroblasts under ER stress revealed several XBP-1-dependent UPR target genes including ERDj4, PSBP2, EDEM, RAMP-4, PDI-P5, and HEDJ (19). In addition to genes involved in UPR, XBP-1 binds to a diverse set of genes in a cell type- and condition-specific manner (20). In vitro studies have shown that cells that have adapted to chronic ER stress by activating UPR become more resistant to subsequent stress, whereas those that have failed to adapt undergo apoptosis (21). When IRE-1 activity was artificially stimulated in human cell lines, cell survival was enhanced upon ER stress in vitro (22), suggesting that the IRE-1/XBP-1 axis affects the cell fate decision between cell death and survival.

XBP1−/− mice die before birth from anemia associated with hypoplasia in the fetal liver (22). Hepatocyte-specific transgenic expression of XBP-1 rescues the embryonic lethality of XBP-1−/− mice, but these animals display abnormalities in other secretory organs such as the pancreas, leading to neonatal death (24). In the immune system, RAG2−/−/− blastocyst complementation revealed an essential role for XBP-1 in the generation of plasma cells. XBP-1−/− B cells develop normally, undergo class-switch recombination, and form germinal centers upon activation, but they fail to secrete IgGs (25). In B cells, transcription of XBP-1u mRNA is directly up-regulated by IL-4 signaling, but splicing to XBP-1s is dependent on additional signals that in-

Bacterial and viral infection

Listeria monocytogenes expressing a secreted form of OVA (LM-OVA) (29) and L. monocytogenes secreting the LCMV gp37 epitope (LM-GP37) were provided by H. Shen (University of Pennsylvania School of Medicine, Philadelphia, PA). LM-OVA and LM-GP37 were grown as previously described (12). LCMV was grown on BHK cells and was titrated on Vero cells. For bacteria infection, mice were injected i.v. with a priming dose of 3000 CFU and a challenge dose of 1−2 × 10^6 CFU. For LCMV infection, a dose of 2 × 10^7 PFU was i.p. injected. Ampicillin treatment of Listeria-infected mice was performed as previously described (30).

Western blot analysis

CD8+ T cells were stimulated for 2 days with 1 μg/ml each of anti-CD3 (145-2C11) and anti-CD28 (37.51) on a dish pretreated with 300 μg/ml anti-hamster IgG (Jackson ImmunoResearch Laboratories), and then main-

RT-PCR and real-time PCR of XBP-1 mRNA

To distinguish XBP-1u and XBP-1s mRNAs, a region encompassing the splicing site of XBP-1 was amplified by RT-PCR with saturating cycles, followed by digestion with PsiI, whose recognition site is located within the 26-nt intron of XBP-1u mRNA (17). For real-time PCR, a primer set was designed to bind upstream of the splicing site to detect total XBP-1 transcripts, including both XBP-1u and XBP-1s mRNA in the same prod-

Retroviral transduction of CD8+ T cells

Retroviruses were produced using Phoenix-E cells transduced with pMitt (MSCV-IRE1-Myr) (34). To distinguish OT-I/XBP-1−/− splenic effector CD8+ T cells from nontreated mice served as a control (0 h). Total RNA from the purified CD8+ T cells was subject to DNA microarray analysis using GeneChip Mouse genome 430 2.0 array (Affymetrix). Target labeling, hybridization, and scanning were performed at the Center for Array Technologies (University of Washington, Seattle, WA).

Flow cytometry

Splenocytes and peripheral blood leukocytes were stained with fluorochromo-conjugated mAbs and then subjected to flow cytometry analysis using a guinea pig complement with streptavidi-
OVA257–264 or LCMV gp33–41 peptides in the presence of brefeldin A (BD Biosciences, GolgiPlug) for 4 h at 37°C. After staining cell surface molecules, intracellular cytokine staining was performed using the Cytofix/Cytoperm kit (BD Biosciences). Fluorochrome-labeled mAbs were obtained from BD Biosciences, eBioscience, and BioLegend. Stained cells were analyzed on FACSCanto II (BD Biosciences), and data were processed by FlowJo software (Tree Star).

Statistical analysis

Statistical differences between groups were examined by one-way ANOVA with Bonferroni’s multiple comparison test or by an unpaired Student’s t test using Prism software (GraphPad). A value of \( p < 0.05 \) was considered statistically significant.

Results

XBP-1 is an IL-2 target gene in CD8\(^+\) T cells in vivo

To understand the mechanisms by which IL-2 signals control CD8\(^+\) T cell responses, we conducted a genome-wide expression analysis. We used injection of IL-2 complex to deliver IL-2 signals to all CD8\(^+\) T cells in vivo. Because STAT5 phosphorylation peaked 1 h after injection of the IL-2 complex (12) (Fig. 1A), gene expression in CD8\(^+\) T cells was compared at 0 h (no treatment), 1 h, and 3 h posttreatment (Fig. 1A). Tables I and II show genes that were up-regulated and down-regulated more than 4-fold at 1

Table I. Genes induced in CD8\(^+\) T cells by IL-2 complex treatment

<table>
<thead>
<tr>
<th>1 h/0 h ( ^a )</th>
<th>3 h/0 h</th>
<th>Gene Title</th>
<th>Gene Symbol</th>
</tr>
</thead>
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<tr>
<td>5.5</td>
<td>5.4</td>
<td>Lymphotixin A</td>
<td>Lxa</td>
</tr>
<tr>
<td>5.3</td>
<td>4.3</td>
<td>Regulator of G protein signaling 1</td>
<td>Rgs1</td>
</tr>
<tr>
<td>5.1</td>
<td>4.7</td>
<td>Cytokine inducible Src homology 2-containing protein</td>
<td>Cish</td>
</tr>
<tr>
<td>4.4</td>
<td>4.1</td>
<td>MAPK kinase kinase 8</td>
<td>Mapkkk8</td>
</tr>
<tr>
<td>3.5</td>
<td>3.0</td>
<td>X-box-binding protein-1</td>
<td>Xbp1</td>
</tr>
<tr>
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<td>3.1</td>
<td>B cell leukemia/lymphoma 2</td>
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<td>3.2</td>
<td>1.6</td>
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<td>Egr1</td>
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<td>2.2</td>
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<td>Axud1</td>
</tr>
<tr>
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<td>2.8</td>
<td>CD69</td>
<td>Cdh6</td>
</tr>
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<td>2.7</td>
<td>2.9</td>
<td>Suppressor of cytokine signaling 1</td>
<td>Socs1</td>
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<td>1.7</td>
<td>Signaling lymphocytic activation molecule family member 1</td>
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<td>2.1</td>
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<td>Adam19</td>
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<td>Myc</td>
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<tr>
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<td>1.2</td>
<td>FBJ osteosarcoma oncogene</td>
<td>Fos</td>
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<td>2.2</td>
<td>2.0</td>
<td>Jun oncogene</td>
<td>Jun</td>
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<td>1.4</td>
<td>Kruppel-like factor 6</td>
<td>Klf6</td>
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<td>1.6</td>
<td>RAS p21 protein activator 2</td>
<td>Ras2</td>
</tr>
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<td>2.0</td>
<td>2.5</td>
<td>Solute carrier family 30, member 4</td>
<td>Slc33a4</td>
</tr>
<tr>
<td>2.0</td>
<td>1.4</td>
<td>NF of ( \alpha ) light polypeptide gene enhancer in B cell inhibitor, ( \zeta )</td>
<td>Nfkbic</td>
</tr>
</tbody>
</table>

\( ^a \) Fold change relative to 0 h is shown as a log2 value.
vs 0 h. A full list can be found in the Gene Expression Omnibus database (GSE11446). In the present study, we focused on XBP-1 because this transcription factor was found to be most up-regulated by IL-2 signals (Table I), and because XBP-1 is known to be critically involved in the differentiation and survival of immune cells, including plasma cells and dendritic cells (25, 27). Real-time PCR confirmed the up-regulation of total XBP-1 transcripts in CD8+ T cells after IL-2 complex treatment (Fig. 1B). XBP-1u and XBP-1s messages can be distinguished in size by RT-PCR combined with restriction enzyme digestion (17). Although IL-2 complex treatment increased the total amount of XBP-1 mRNA in CD8+ T cells, it did not induce the conversion of XBP-1u to XBP-1s by 3 h (Fig. 1C), at which point, STAT5 phosphorylation and XBP-1 induction had already begun to fade (Fig. 1, A and B). IL-2 complex treatment in vivo activates STAT5 signaling in CD8+ T cells as early as 15 min after treatment (12), suggesting the direct action of IL-2 signals on XBP-1 mRNA induction in CD8+ T cells in vivo. In support of this idea, we found that in vitro stimulation with IL-2 up-regulated XBP-1 mRNA in purified CD8+ T cell blasts (Fig. 1D), which formally excluded the possibility of a secondary effect of the complex through another cell type expressing the IL-2R. In addition to IL-2, IL-4 and IL-15 also increased total XBP-1 mRNA levels in CD8+ T cells in vitro (Fig. 1D). These results demonstrate that IL-2 signals induce XBP-1u mRNA in CD8+ T cells, but they do not generate the spliced, transcriptionally active form of XBP-1.

**TCR ligation induces the generation of XBP-1s in vitro**

We next tested whether TCR cross-linking induces the generation of XBP-1s. CD8+ T cells were activated by plate-bound anti-CD3 and anti-CD28 mAbs, and the CD8+ T cell blasts maintained for several days in IL-2-containing medium. No obvious increase of XBP-1s mRNA was detected during culture with IL-2 alone (Fig. 2A at 0 h and an additional 24-h culture without TCR ligation). In contrast, restimulation with anti-CD3 and anti-CD28 mAbs clearly induced splicing and XBP-1s accumulation in CD8+ T cell blasts (Fig. 2A). This observation was also confirmed at the protein level by Western blot (Fig. 2B). We noted that XBP-1s gradually disappeared 8–24 h after TCR stimulation (Fig. 2, A and B). These results suggest that whereas IL-2 up-regulates XBP-1 transcripts, antigen stimulation through the TCR is required for the generation of transcriptionally active XBP-1s.

**Activation of the IRE-1/XBP-1 pathway in effector CD8+ T cells during acute infection**

Acute infection with viruses or bacteria induces vigorous expansion of Ag-specific CD8+ T cells. We hypothesized that these dynamic CD8+ T cell responses involving both inflammatory and antigenic stimuli would activate the IRE-1/XBP-1 pathway. After LCMV infection, most of the CD8+ T cell population consists of LCMV-specific effector cells 8 days postinfection (p.i.) (36). Total CD8+ T cells in the spleen of LCMV-infected C57BL/6J mice were examined 8 days p.i. for XBP-1 mRNA splicing. As shown in Fig. 3A, whereas CD8+ T cells from uninfected mice contained no or little XBP-1s mRNA, LCMV infection clearly induced IRE-1-mediated splicing and generated XBP-1s mRNA in CD8+ T cells.

To investigate XBP-1 splicing status in different subsets of effector CD8+ T cells, we used transgenic mice that ubiquitously express an XBP-1 splicing reporter, referred to as ER stress-activated indicator, ERAI (28). In this system, Venus (a GFP variant) is expressed only in cells that contain XBP-1 splicing activity (Fig. 3B). Consistent with the previous report showing that dendritic cells constitutively activate the XBP-1 pathway (27), unstimulated splenic CD11c+ dendritic cells from the ERAI transgenic mice were positive for Venus-GFP (Fig. 3C). Before infection, CD8+ T cells from various organs of the ERAI mice showed no detectable signal of XBP-1 splicing activity (data not shown). However, gp33-specific effector cells from LCMV-infected

![FIGURE 2.](http://www.jimmunol.org/) **TCR stimulation induces splicing of XBP-1 message.** Purified CD8+ T cells were activated with plate-bound anti-CD3 and anti-CD28 mAbs, and maintained in IL-2-containing medium. These CD8+ T cell blasts were restimulated with anti-CD3 and anti-CD28 mAbs for the indicated times. A. Splicing of XBP-1u mRNA detected by RT-PCR and PstI digestion. B. Western blot analysis detecting both forms of XBP-1. A nonspecific band as a loading control is indicated (+).
FIGURE 3. Acute infection activates the IRE-1/XBP-1 pathway in CD8+ T cells. A, Splicing status of XBP-1 mRNA in total CD8+ T cells purified from uninfected or LCMV-infected mice at day 8 p.i. Each lane represents an individual animal. B, The XBP-1-splicing reporter system. After removal of the 26-nt intron, the Venus-GFP cDNA becomes in-frame in the reporter construct. The reporter construct lacks the DNA binding domain (DBD) to prevent a dominant negative action of the truncated XBP-1 protein (28). TA, transcriptional activating domain. C, Venus-GFP levels in splenic dendritic cells of ERAI transgenic mice. Plots are gated on CD3+ CD19+ CD11c+ population. D, WT and ERAI transgenic (Tg) reporter mice were infected with LCMV, and splenocytes stained with Dbgp33 tetramer on day 8. Plots are gated on CD8+ cells. The percentage of gp33-specific population within CD8+ cells (top) is shown. Venus-GFP levels in gated gp33-specific CD8+ T cells (bottom) are shown. E, Mean fluorescence intensity (MFI) of Venus-GFP within KLRG1low or KLRG1high gp33-specific CD8+ T cell populations at day 8 p.i. Results indicate the mean ± SEM (n = 3–4 mice). F, OT-I cells expressing the XBP-1 splicing reporter or mock construct were obtained from mice reconstituted with retrovirally transduced bone marrow cells. These OT-I cells were transferred to host mice that were subsequently infected with 3000 CFU LM-OVA. Some infected animals were rested for 63 days, then given a high dose (1 × 10^6 CFU) of LM-OVA, and analyzed 3 days later (d63+3). Plots are gated on OT-I cells with Thy1.1 transduction marker. The percentage of the gated OT-I cells within each quadrant is shown. G, Splicing status of XBP-1 mRNA in naive CD44low OT-I cells (N) or OT-I cells from mice that had received 10^4 naive OT-I cells and were then infected with LM-OVA. H, Splicing status of XBP-1 mRNA in naive CD44low P14 cells (N) or P14 cells from LCMV-infected mice that had received 10^4 naive P14 cells. Some previously infected mice were challenged with 2 × 10^6 CFU of LM-GP33, and analyzed after 4 days (d36+4).
ERAI mice demonstrated XBP-1 splicing activity 8 days p.i. (Fig. 3D). When costained with KLRG1, the splicing activity was detectable in both KLRG1low and KLRG1high populations, but was significantly enriched in the KLRG1high terminal effector population (Fig. 3E).

Splicing of XBP-1u mRNA in effector CD8+ T cells was also triggered during a bacterial infection with LM-OVA (Fig. 3, F and G). Similar to LCMV infection, the splicing activity was skewed to the KLRG1high population in effector CD8+ T cells specific for OVA (Fig. 3F). Time-course analyses showed that XBP-1 mRNA splicing mainly occurred during primary expansion (~day 7 or 8 p.i.) and secondary expansion after rechallenge of Ag-specific CD8+ T cells (Fig. 3, F–H). These results demonstrated that the IRE-1/XBP-1 splicing response is activated in effector CD8+ T cells during acute infection, and suggest a correlation between high levels of XBP-1s and terminal differentiation of effector CD8+ T cells.

**Overexpression of XBP-1s enhances KLRG1 levels on effector CD8+ T cells**

Enrichment of the XBP-1 splicing activity in KLRG1high effector cells suggested that overexpression of XBP-1s would promote terminal differentiation. OT-I/RAG1−/−CD8+ T cells were stimulated in vitro to allow retroviral transduction of various forms of XBP-1.
FIGURE 5. Loss-of-function of XBP-1 results in diminished KLRG1high effector CD8+ T cell population. A, OT-I/RAG1−/− CD8+ T cells were transduced with a dominant negative form of XBP-1 (dnXBP-1) and transferred to host mice, followed by infection with LM-OVA. KLRG1 levels on the transduced cells (day 7 p.i.) in the blood are shown. Graphic representation of mean fluorescence intensity (MFI) of KLRG1 (right). Results indicate the mean ± SEM (n = 5 mice each). B, WT or XBP-1−/− bone marrow chimeric mice were infected with LCMV. On day 8, gp33-specific CD8+ T cells in the blood were detected by tetramer staining. Percentage of Dbgp33 tetramer-positive within the donor CD8+ population (top) gated on CD229.1+ donor bone marrow-derived CD8+ cells is shown. KLRG1 and CD127 levels on gp33-specific CD8+ T cells are shown. The percentage within the donor gp33-specific CD8+ T cells (bottom) is indicated. C, Graphic representation of data in B with the mean ± SEM for n = 8–10 mice.

XBP-1. Transduced OT-I/RAG1−/− cells, marked by Thy1.1 expression, were sorted and adoptively transferred (10⁴ cells/mouse) into Thy1.1-negative host mice that were subsequently infected with 3000 CFU LM-OVA. After infection, XBP-1u, XBP-1s, and mock-transduced OT-I/RAG1−/− cells expanded and contracted with similar kinetics (Fig. 4, A and B). Cytokine expression in these OT-I/RAG1−/− effector cells at day 7 p.i. following 4 h of peptide stimulation was not significantly affected (data not shown). However, KLRG1 levels on XBP-1s transduced effector OT-I/RAG1−/− cells were clearly higher at day 7 p.i. than on mock-transduced cells, and the higher expression levels were maintained thereafter (Fig. 4C). XBP-1u overexpression had no such enhancing effect. Following Listeria infection, shortening the infectious period by antibiotic treatment hampers the terminal differentiation of effector CD8+ T cells (4, 30). Accordingly, ampicillin treatment starting at 24 h p.i. diminished the differentiation of mock-transduced OT-I/RAG1−/− cells into the KLRG1high population, and this effect was reversed by retroviral expression of XBP-1s (Fig. 4D). In contrast to retroviral expression of T-bet, which is known to regulate effector CD8+ T cell differentiation (4, 37), KLRG1 expression was not induced by XBP-1s expression itself during 10 days of in vitro culture with IL-2 (Fig. 4E). These results suggest that XBP-1s potentiates KLRG1 levels on effector CD8+ T cells but does not directly control the expression.

Down-regulation of XBP-1 limits the development of KLRG1high effector CD8+ T cells

We next tried to suppress XBP-1 levels by expressing a dominant negative form of XBP-1 (dnXBP-1) that possesses the DNA binding domain but lacks the transactivating domain (27, 34). Retroviral-mediated expression of dnXBP-1 significantly suppressed KLRG1 levels on transduced effector OT-I/RAG1−/− cells at day 7 after infection with LM-OVA (Fig. 5A). To further investigate the role of XBP-1 in CD8+ T cells, we made use of XBP-1−/− cells from Rag2−/− blastocyst chimeras (38). Bone marrow chimeric mice containing XBP-1−/− CD8+ T cells were generated using cells from XBP-1−/− Rag2−/− blastocyst chimeras (25, 27). In these bone marrow chimeras, XBP-1−/− CD8+ T cells were detected by the CD229.1 allelic marker, which is not expressed on host cells of C57BL/6 origin. Bone marrow cells from 129WT/Rag2−/− blastocyst chimeras were used to generate control bone marrow chimeras. These bone marrow chimeras were infected with LCMV, and effector CD8+ T cell populations were examined 8 days later. Although similar levels of the gp33-specific response were observed by WT and knockout cells, XBP-1−/− CD8+ T cells formed a proportionally smaller KLRG1highCD127low population than did the WT cells (Fig. 5, B and C). The lack of XBP-1 did not severely impact cytokine production during a 4-h stimulation period (data not shown). These results suggest that XBP-1 is not required to mount a robust CD8+ T cell response to acute infection, but is required for the optimal differentiation of effector CD8+ T cells.

Discussion

In the present study, we demonstrated that the IRE-1/XBP-1 splicing machinery, known to be involved in the UPR, is activated in effector CD8+ T cells during acute infection. IL-2 signals (as well as IL-4 and IL-15 in vitro) up-regulate XBP-1 mRNA in CD8+ T cells.
cells in vivo, whereas TCR stimulation induces splicing and the generation of XBP-1s in vitro. Previous gene profiling studies showed that XBP-1 mRNA is up-regulated by IL-2 in vitro in human PBMC and mouse CD4+ T cells (39–42), suggesting a common mechanism of XBP-1 induction in T cells. Furthermore, we showed that XBP-1s contributes to driving terminal differentiation of effector CD8+ T cells. IRE-1 is an ER resident kinase/RNase that senses ER stress and is responsible for splicing XBP-1 mRNA (16, 17). When the UPR is pharmacologically induced in human cell lines, IRE-1 activities are quickly attenuated, compared with the other two pathways of UPR (22). Interestingly, under persistent ER stress, cells survive longer when the IRE-1/RNase activity is artificially sustained (22). Thus, the IRE-1/XBP-1 pathway affects cell fate decisions between cytoprotective and proapoptotic outcomes by the UPR. We consistently observed that the level of XBP-1s waned 8–24 h after anti-CD3 and anti-CD28 mAb stimulation (Fig. 2), suggesting that CD8+ T cells use a similar mechanism to turn this pathway off. Our gain-of-function and loss-of-function studies suggested a positive regulatory role for XBP-1s in KLRG1 expression. Unlike T-bet, however, overexpression of XBP-1s itself did not induce KLRG1 expression on CD8+ T cells in vitro (Fig. 4E). In addition, XBP-1s did not significantly affect KLRG1 levels on responding CD8+ T cells on day 5 following infection (Fig. 4C), when the cell fate commitment to terminal effectors vs memory precursor cells has already begun (4, 5). It has been reported that KLRG1 expression is associated with extensive cell division (43). Therefore, we assume that XBP-1s supports, rather than directs, differentiation of CD8+ T cells toward end-stage effector cells, possibly by affecting cell survival or proliferation.

The induction of XBP-1 mRNA and regulation of the unique splicing in CD8+ T cells appeared analogous to that in B cells. XBP-1 mRNA is induced in B cells by IL-4, but splicing to XBP-1s mRNA requires further stimulation with LPS and/or CD40 engagement (26). XBP-1 has a nonredundant role in the differentiation of B cells into plasma cells (25, 26), whereas one of the other arms of UPR, the PERK/eIF2α pathway, is not significantly involved in this process (44, 45). Our DNA microarray data showed that ATF4 mRNA, which is downstream of the PERK/eIF2α pathway, is also up-regulated in CD8+ T cells after IL-2 complex treatment (Table I). In future, it will be intriguing to examine the role of other pathways of the UPR in CD8+ T cell responses.

Virus infection activates the UPR in host cells due to the massive production of viral proteins (46), raising the question of whether this is happening in our in vivo studies. LCMV can infect T cells, but at a very low rate (<1%), as judged by intracellular staining for LCMV nucleoprotein (47). Furthermore, splicing of XBP-1 mRNA was also evident in CD8+ T cells after bacterial infection (Fig. 3, F and G). Therefore, it is not likely that the activation of XBP-1 splicing machinery in CD8+ T cells is a consequence of direct infection. In vitro experiments demonstrating the splicing of XBP-1 mRNA after TCR ligation (Fig. 2) further reinforce this conclusion.

Does the activation of the XBP-1 splicing machinery in CD8+ T cells represent induction of the UPR? Effector CD8+ T cells produce many cytokines and cytotoxic molecules, and calcium release from the ER is induced upon TCR ligation, both of which potentially cause ER stress. However, a Gene Expression Omnibus database search (GSE10239 (5), and GSE9650 (48)) and our preliminary real-time PCR analyses (data not shown) revealed that UPR target genes such as Bip (Hspa5), CHOP (Ddit3), ERdj4 (Dnab9), and EDEM (Edem1) were not significantly increased in CD8+ T cells during the course of LCMV infection. Furthermore, in contrast to our expectation, cytokine production by CD8+ T cells during a brief period of Ag stimulation in vitro was not changed by the forced expression of XBP-1s or a null mutation of XBP-1 (data not shown). XBP-1 may be required for the sustained production of these effector cytokines, similar to its role in μ chain production in B cells (49). UPR induction in the course of plasma cell differentiation is well documented (14, 50). Despite the almost complete block of plasma cell differentiation in the absence of XBP-1 (25), however, Ivakoshi et al. (26) described that induction of ER chaperones including GRP78 (Bip) and GRP94 is only slightly impaired in XBP-1−/− B cells. Furthermore, Tirosh et al. (49) reported only a minor role for XBP-1 in ER quality control of primary B cells. ChIP-on-ChIP analysis of XBP-1 using different cell types identified a number of cell type- and condition-specific target genes (20). These observations suggest an as yet unknown role for XBP-1 beyond that in the UPR, which may be applicable to CD8+ T cell responses. It is also possible that UPR target gene products are simply maintained at pre-existing levels by XBP-1s during the dramatic burst of proliferation of Ag-specific CD8+ T cells resulting in pathogen infection (1).

In summary, we have demonstrated the activation of IRE-1/XBP-1 splicing machinery in CD8+ T cells in a physiological context. These findings also suggest that the stress control pathway in the ER contributes to the differentiation of effector CD8+ T cells in vivo.

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Disclosures
The authors have no financial conflict of interest.

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