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A Role for the Transcriptional Repressor Blimp-1 in CD8$^+$ T Cell Exhaustion during Chronic Viral Infection

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SUMMARY

T cell exhaustion is common during chronic infections and can prevent optimal immunity. Although recent studies have demonstrated the importance of inhibitory receptors and other pathways in T cell exhaustion, the underlying transcriptional mechanisms are unknown. Here, we define a role for the transcription factor Blimp-1 in CD8$^+$ T cell exhaustion during chronic viral infection. Blimp-1 repressed key aspects of normal memory CD8$^+$ T cell differentiation and promoted high expression of inhibitory receptors during chronic infection. These cardinal features of CD8$^+$ T cell exhaustion were corrected by conditionally deleting Blimp-1. Although high expression of Blimp-1 fostered aspects of CD8$^+$ T cell exhaustion, haploinsufficiency indicated that moderate Blimp-1 expression sustained some effector function during chronic viral infection. Thus, we identify Blimp-1 as a transcriptional regulator of CD8$^+$ T cell exhaustion during chronic viral infection and propose that Blimp-1 acts as a transcriptional rheostat balancing effector function and T cell exhaustion.

INTRODUCTION

After acute viral infections, antigen-specific CD8$^+$ T cells can undergo a memory differentiation program that results in the development and maintenance of robust, functional memory CD8$^+$ T cells. Once a pathogen is cleared, memory CD8$^+$ T cell differentiation follows a characteristic differentiation program in which a subset of IL-7Rα^{hi} effector CD8$^+$ T cells persists into the memory pool, increases expression of IL-7Rα and lymphoid homing molecules such as CD62L and CCR7, and gains the ability to produce IL-2 (Kaech and Wherry, 2007; Williams and Bevan, 2007). These memory CD8$^+$ T cells also acquire several cardinal properties such as the ability to rapidly respond and reactivate effector functions upon antigen re-exposure, high proliferative potential, and long-term antigen-independent maintenance via IL-7- and IL-15-driven self-renewal (Kaech and Wherry, 2007; Williams and Bevan, 2007).

In contrast to acute infections, during chronic viral infections virus-specific CD8$^+$ T cells undergo an altered pattern of differentiation and become exhausted. CD8$^+$ T cell exhaustion is a transcriptionally altered state of T cell differentiation distinct from functional effector or memory CD8$^+$ T cells (Wherry et al., 2007). Although some persisting infections such as Epstein Barr Virus (EBV) and cytomegalovirus (CMV) induce functional T cells that control these infections in healthy people, many other chronic infections in animal models and humans, particularly those with sustained viral replication, are associated with T cell exhaustion (Shin and Wherry, 2007). In these situations, exhausted CD8$^+$ T cells undergo a hierarchical loss of function, ultimately resulting in virus-specific CD8$^+$ T cells with severely compromised effector function, and in some cases these cells are physically deleted (Fuller and Zajac, 2003; Wherry et al., 2003). In addition, unlike normal memory CD8$^+$ T cells generated after acute infections, during chronic infections virus-specific CD8$^+$ T cells remain IL-7Rα^{lo}, IL-15Rβ^{lo}, CD62L^{lo}, and IL-2^{lo} (Shin and Wherry, 2007). These virus-specific CD8$^+$ T cells have major defects in homeostatic proliferation and often become dependent on persisting antigen rather than IL-7 and IL-15 for maintenance (Shin et al., 2007).

Another cardinal feature of exhausted CD8$^+$ T cells is the sustained expression of multiple inhibitory receptors (Blackburn et al., 2009; Kaufmann et al., 2007; Nakamoto et al., 2008; Wherry et al., 2007). Whereas the precise role of inhibitory receptors in the initiation of CD8$^+$ T cell exhaustion remains unclear, considerable evidence indicates that the expression of these receptors is important for regulating multiple functional aspects of CD8$^+$ T cell exhaustion once a chronic infection is established. The programmed death 1 (PD-1) pathway has received considerable attention because exhausted CD8$^+$ T cells have substantially higher expression of this molecule, and blockade of this pathway in vivo reinvigorates antiviral CD8$^+$ T cell responses (Barber et al., 2006; Sharpe et al., 2007; Velu et al., 2009). However, additional pathways, including lymphocyte activation gene 3 (LAG-3), also have a major role in limiting the effectiveness of exhausted CD8$^+$ T cells during chronic viral infection (Blackburn et al., 2009). Other known or potential inhibitory pathways that have also been associated with T cell dysfunction during chronic viral infections include 2B4, CD160, PirB, GP49B, CTLA-4, and Tim3 (Blackburn et al., 2009; Jones et al., 2008; Kaufmann et al., 2007; Nakamoto et al., 2008; Wherry et al., 2007) and different inhibitory receptors may regulate distinct aspects of functional exhaustion (Blackburn et al., 2009). Thus, the factors controlling expression of inhibitory receptors could represent a fundamental control mechanism for T cell exhaustion.

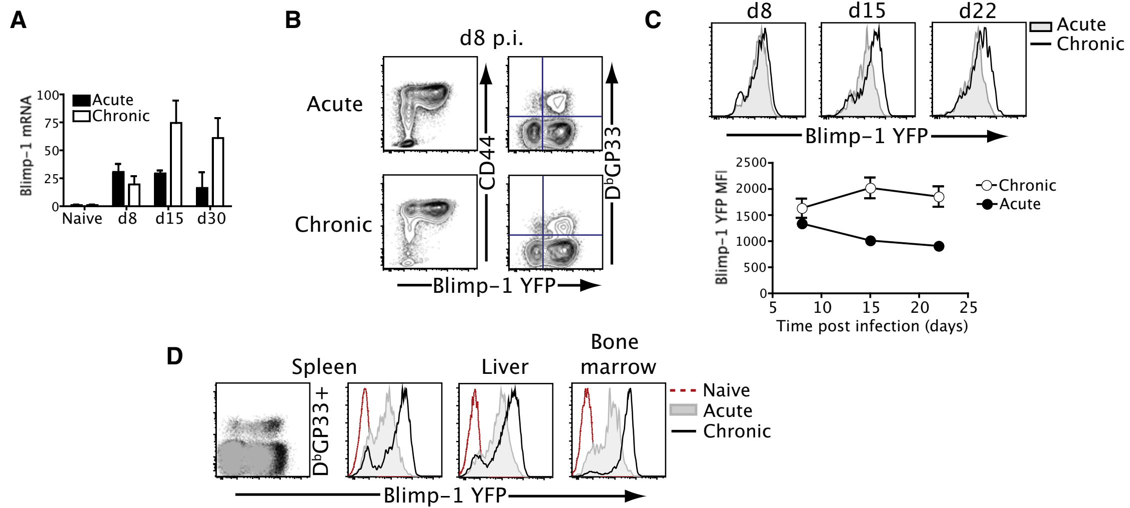

Figure 1. Blimp-1 Was Highly Expressed in Exhausted Antigen-Specific CD8+ T Cells during Chronic Viral Infection

(A) Blimp-1 message by qRT-PCR. DbGP33+CD8+ T cells were sorted by FACS from LCMV Arm (acute)- or clone 13 (chronic)-infected mice at the indicated d.p.i. Naive controls were CD44lo CD8+ T cells sorted from Arm immune. Error bars show range of expression in triplicate wells.

(B) Blimp-1 YFP reporter expression in CD8+ T cells on d8 p.i. with LCMV Arm or clone 13. Plots are gated on total CD8+ T cells.

(C) Kinetics of Blimp-1 YFP reporter expression after LCMV Arm or clone 13 infection. Histograms are gated on DbGP33+ CD8+ T cells from the blood of LCMV Arm (shaded) or LCMV clone 13 (open) infection. Data points in graph show Blimp-1 YFP reporter MFI of DbGP33+ CD8+ T cells from LCMV Arm (black circles) or LCMV clone 13 (white circles) at the indicated d.p.i. Error bars are standard error of the mean (SEM). n = 2–3 mice per time point.

(D) Blimp-1 YFP reporter MFI in tissues 30 d.p.i. with LCMV Arm or clone 13. Dot plot is gated on total CD8+ T cells. Histograms are gated on DbGP33+ CD8+ T cells from LCMV Arm (shaded) or LCMV clone (open) infection. Open red histograms show CD44lo CD8+ T cells from LCMV Arm infection.

Despite recent work on inhibitory receptors and on the transcriptional profiles of exhausted CD8+ T cells (Wherry et al., 2007), the fundamental transcriptional mechanisms underlying this type of T cell dysfunction are unknown. In the present study, we have examined the role of the transcriptional repressor Blimp-1 in CD8+ T cell exhaustion during chronic viral infection in mice. Blimp-1 is a zinc finger-containing transcriptional repressor perhaps best known for governing fate decisions in memory B cell differentiation. In the germinal center, expression of Blimp-1 promotes terminal differentiation of plasma cells and also represses the transcriptional program of memory B cells (Calame, 2006; Shaffer et al., 2002; Shapiro-Shelef et al., 2003). Blimp-1 also has been shown to regulate fate decisions and cellular differentiation in other hematopoietic and nonhematopoietic cells (John and Garrett-Sinha, 2008). Here, we show that Blimp-1 has an important role as a transcriptional regulator of CD8+ T cell exhaustion. Elevated expression of Blimp-1 in virus-specific exhausted CD8+ T cells during chronic viral infection was associated with repression of memory T cell differentiation and elevated expression of inhibitory receptors. Both of these features of CD8+ T cell exhaustion were reversed when Blimp-1 was conditionally deleted. However, haploinsufficient mice controlled chronic viral infection better than either WT or full conditionally deficient mice. Thus, our studies indicate that Blimp-1 is a transcriptional regulator of CD8+ T cell exhaustion and suggest a model in which Blimp-1 is a transcriptional rheostat regulating the balance between CD8+ T cell effector function when moderately expressed and CD8+ T cell exhaustion when highly expressed.

RESULTS

Blimp-1 Is Overexpressed in Virus-Specific CD8+ T Cells during Chronic Viral Infection

To investigate the role of Blimp-1 in CD8+ T cells during chronic viral infection, we used lymphocytic choriomeningitis virus (LCMV). LCMV infection of adult mice is a well-established model with which to examine virus-specific T cell differentiation during acute versus chronic infection. Infection with the Armstrong strain of LCMV (Arm) results in an acute infection with clearance of infectious virus by day 8–10 postinfection (p.i.) and generation of functional effector and memory T cells (Wherry et al., 2003). In contrast, infection with the clone 13 strain of LCMV results in a chronic infection with viremia for 2–3 months, long-term viral persistence in some tissues and T cell exhaustion (Ahmed et al., 1984; Wherry et al., 2003). To determine the kinetics of Blimp-1 expression in virus-specific CD8+ T cells during acute versus chronic LCMV infection, we first used quantitative RT-PCR (qRT-PCR) (Figure 1A). DbGP33-specific CD8+ T cells were sorted from the spleens of Arm or clone 13 infected mice on day 8, 15, and 30 p.i. and Blimp-1 mRNA expression was examined (Figure 1A). At day 8 p.i., Blimp-1 expression was upregulated to a similar degree in DbGP33-specific CD8+ T cells from either Arm- or clone 13-infected mice (Figure 1A). After the first week of infection, Blimp-1 mRNA decreased modestly in DbGP33-specific CD8+ T cells from LCMV Arm infected mice. In contrast, Blimp-1 was highly upregulated in virus-specific CD8+ T cells from clone 13 infected mice by day 15 p.i. and remained highly expressed for at least 1 month (Figure 1A).

Blimp-1 expression in virus-specific CD8$^+$ T cells was also examined during acute versus chronic LCMV infection with a Blimp-1 YFP reporter mouse. After infection with either Arm or clone 13, YFP expression in the reporter mice was elevated in CD44^{hi} CD8$^+$ T cells, but remained low in naive (CD44^{lo}) CD8$^+$ T cells (Figure 1B). DbGP33-tetramer+ CD8$^+$ T cells were almost uniformly YFP+ (i.e., Blimp-1+) at day 8 p.i. (Figure 1B) and the MFI of YFP was similar in DbGP33-specific CD8$^+$ T cells between acute and chronic viral infection (Figure 1C). However, after day 8 p.i., YFP MFI increased in DbGP33-specific CD8$^+$ T cells during chronic LCMV infection, but decreased after day 8 subsequent to acute infection (Figure 1C). Blimp-1-driven YFP expression was not limited to a specific tissue microenvironment and was elevated in antigen-specific CD8$^+$ T cells in both lymphoid and nonlymphoid tissues during chronic viral infection (Figure 1D). There were also differences in Blimp-1 YFP expression in different epitope-specific CD8$^+$ T cell populations during chronic infections. DbNP396-specific CD8$^+$ T cells undergo exhaustion early during chronic infection and are often physically deleted (Fuller and Zajac, 2003; Wherry et al., 2003). These DbNP396-specific CD8$^+$ T cells expressed higher Blimp-1 YFP than the DbGP33-specific CD8$^+$ T cells that also become exhausted but persist during chronic infection (Figure S1 available online). Thus, Blimp-1 expression was substantially higher in antigen-specific CD8$^+$ T cells during chronic viral infection than after acute viral infection. Moreover, the pattern of Blimp-1 expression suggested a correlation between Blimp-1 expression and T cell dysfunction and/or terminal differentiation.

Conditional Deletion of Blimp-1 in Activated CD8 T Cells with Granzyme B-Cre

Blimp-1 has recently been described to play a role in T cell activation and homeostasis (Kallies et al., 2006; Martins et al., 2006). On the basis of the substantial increase in Blimp-1 expression during chronic viral infection, we questioned whether Blimp-1 might have a particular relevance during CD8$^+$ T cell exhaustion. To examine this issue, we used conditional deletion of *Prdm1*, the gene encoding Blimp-1. To avoid any potential complications due to the action of Blimp-1 in the early events of T cell activation, we crossed *Prdm1$^{fl/fl}$* mice (Shapiro-Shelef et al., 2003) to mice with cre recombinase expression driven by the human granzyme B promoter (gzmB-cre) (Jacob and Baltimore, 1999). Hereafter, we refer to these *Prdm1$^{fl/fl}$* × gzmB-cre mice as gzmB-cre *Prdm1$^{fl/fl}$*. To determine when gzmB-cre was active after infection, we crossed the gzmB-cre mice to Rosa26-f-stop-f-YFP mice, which have a floxed stop site upstream of the gene encoding YFP in the Rosa26 locus. Granzyme B is expressed in activated CD8$^+$ T cells 1–2 days after activation (Chang et al., 2007) and accordingly, YFP fluorescence was present in activated CD25$^+$ CD8$^+$ T cells by 3 days after LCMV clone 13 infection of gzmB-cre × Rosa26-f-stop-f-YFP mice (Figure 2A). By day 6 p.i., YFP was expressed by the majority of activated, CD44^{hi} and tetramer+ CD8$^+$ T cells, indicating cre-mediated recombination had occurred in most responding CD8$^+$ T cells at this time (Figure 2A, data not shown), although in the gzmB-cre *Prdm1$^{fl/fl}$* mice, it remains possible that some Blimp-1 mRNA and/or protein could persist temporarily after gene deletion.

Blimp-1 Deficiency Alters Virus-Specific CD8$^+$ T Cell Differentiation during Chronic Viral Infection

GzmB-cre *Prdm1$^{fl/fl}$* and wild-type (WT) littermates were infected with LCMV clone 13, and viral control and T cell responses were examined. Through the first ~20–30 days of infection, the viral load in the serum and tissues was similar between WT and gzmB-cre *Prdm1$^{fl/fl}$* mice (Figure 2B). The frequency of antigen-specific CD8$^+$ T cells in the blood was also similar between the gzmB-cre *Prdm1$^{fl/fl}$* and WT groups, although the contraction phase appeared delayed in the gzmB-cre *Prdm1$^{fl/fl}$* mice (Figure 2C). Spleens of gzmB-cre *Prdm1$^{fl/fl}$* mice tended to contain higher absolute numbers of CD8$^+$ T cells specific for three different LCMV epitopes compared to WT mice at day 30 p.i. (Figure 2C). There were also more DbGP33-specific CD8$^+$ T cells in the inguinal lymph nodes of the gzmB-cre *Prdm1$^{fl/fl}$* mice, but similar numbers in the bone marrow and liver of gzmB-cre *Prdm1$^{fl/fl}$* and WT mice (Figure 2C). Among the different virus-specific CD8$^+$ T cell populations examined, we observed a larger increase in the number DbNP396-specific CD8$^+$ T cells in the gzmB-cre *Prdm1$^{fl/fl}$* mice over WT mice (3-fold) compared to DbGP33 or DbGP276-specific CD8$^+$ T cell populations (2.2-fold and 1.8-fold, respectively), suggesting that deletion of Blimp-1 had the greatest impact on the accumulation of the most severely exhausted CD8$^+$ T cells (Figure 2C).

The phenotype of conditionally Blimp-1-deficient antigen-specific CD8$^+$ T cells was distinct from WT antigen-specific CD8$^+$ T cells. Many of the gzmB-cre *Prdm1$^{fl/fl}$* antigen-specific CD8$^+$ T cells expressed CD127 and CD62L, two molecules associated with memory CD8$^+$ T cell differentiation after acute infections that are not normally expressed by exhausted CD8$^+$ T cells (Figure 2D). Even DbNP396-specific CD8$^+$ T cells expressed CD127 and CD62L in the absence of Blimp-1 (Figure S2A). Despite the expression of some memory-like markers by conditionally Blimp-1-deficient CD8$^+$ T cells, the gzmB-cre *Prdm1$^{fl/fl}$* CD8$^+$ T cells had only modest changes in cytokine production. IFN-γ production was similar between gzmB-cre *Prdm1$^{fl/fl}$* and WT CD8$^+$ T cells and MIP-1α was slightly reduced in the gzmB-cre *Prdm1$^{fl/fl}$* CD8$^+$ T cells (Figure 2E). Although production of TNF was slightly elevated in the conditionally Blimp-1-deficient T cells, both DbGP33-specific and DbNP396-specific CD8$^+$ T cells from the gzmB-cre *Prdm1$^{fl/fl}$* mice were still considerably less functional than memory CD8$^+$ T cells from immune mice, in which 80%–95% of the antigen-specific CD8$^+$ T cells coproduced IFN-γ and TNF (Figure 2E and Figure S2) (Wherry et al., 2003). In agreement with previous reports (Martins et al., 2008; Martins et al., 2006), IL-2 production was elevated in the gzmB-cre *Prdm1$^{fl/fl}$* antigen-specific CD8$^+$ T cells to a degree that was comparable to memory CD8$^+$ T cells after acute viral infection (Figure 2E and Figure S2). Thus, loss of Blimp-1 in antigen-specific CD8$^+$ T cells with gzmB-cre did not alter the initial viral load during chronic infection nor did it substantially improve effector cytokine production. Conditional deletion of Blimp-1 did, however, resulted in a trend toward increased numbers of antigen-specific CD8$^+$ T cells, restored some key aspects of normal memory CD8$^+$ T cell differentiation, including CD127 and CD62L expression, and led to the partial restoration of antigen-specific CD8$^+$ T cell populations that were otherwise terminally differentiated and deleted during chronic viral infection (e.g., DbNP396).

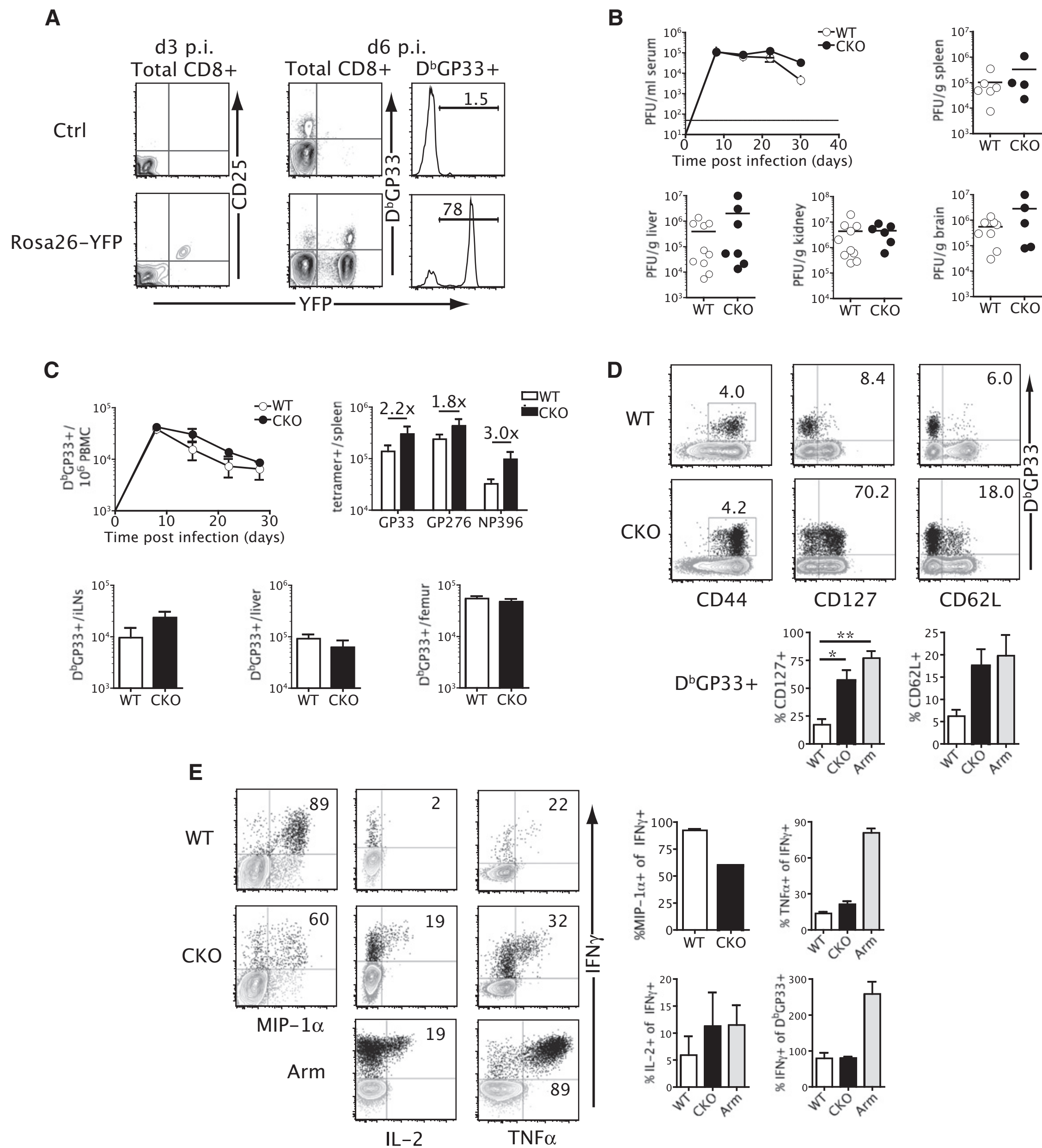

Figure 2. CD8+ T Cell Responses in Blimp-1 Conditional Knockout Mice during Chronic Viral Infection

(A) Efficiency of granzyme B-cre-mediated gene deletion. Rosa26-f-stop-f-YFP x gzmB-cre mice or WT littermates were infected with LCMV clone 13. CD8+ T cells were analyzed for YFP expression at day 3 (blood) and day 6 (spleen) p.i. Plots are gated on total CD8+ T cells and histograms are gated on DbGP33+ CD8+ T cells. Numbers above gates in the histograms indicate the percentage of DbGP33+ CD8+ T cells that are YFP+.

(B) Viral loads in multiple tissues in WT and gzmB-cre *Prdm1*$^{fl/fl}$ mice (CKO). GzmB-cre *Prdm1*$^{fl/fl}$ mice and WT littermates were infected with LCMV clone 13 and analyzed at day 30 p.i. unless otherwise noted. Viral titers were measured by plaque assay at the indicated time points in the serum and tissues. Data are representative of four independent experiments. Error bars in serum titers represent SEM. n = 3–10 mice per time point.

(C) Absolute number of antigen-specific CD8+ T cells in WT and gzmB-cre *Prdm1*$^{fl/fl}$ mice. The frequency of DbGP33+ CD8+ T cells was measured in the blood at indicated time points. The absolute number of tetramer+ CD8+ T cells was measured in each tissue at day 30 p.i. Numbers above spleen graph indicate the difference in number between gzmB-cre *Prdm1*$^{fl/fl}$ and WT for each tetramer+ population. Error bars represent SEM, and n = 4–8 for each tissue. Data represent three independent experiments.

(D) Phenotype of WT and gzmB-cre *Prdm1*$^{fl/fl}$ antigen-specific CD8+ T cells. Plots are gated on total CD8+ T cells in the spleen. Numbers in left-hand plots show percentage of total CD8+ T cells that are DbGP33+. Numbers in the center and right-hand plots indicate the percentage of DbGP33+ CD8+ T cells that are positive for each marker. Graphs represent two independent experiments; “Arm” indicates marker expression for memory CD8+ T cells from Arm immune mice (~day 30 p.i.). Error bars are SEM. For CD127, *p = 0.04, **p = 0.002 by Student’s t test.

(E) Effector function of WT and gzmB-cre *Prdm1*$^{fl/fl}$ antigen-specific CD8+ T cells compared to memory CD8+ T cells (day 30+ Arm immune). Splenocytes were stimulated with GP33 peptide for 5 hr. Plots are gated on CD8+ T cells. Numbers in each plot show the percent of IFNγ producing CD8+ T cells that also produced the second indicated cytokine. Numbers are graphed to the right. All error bars represent SEM.

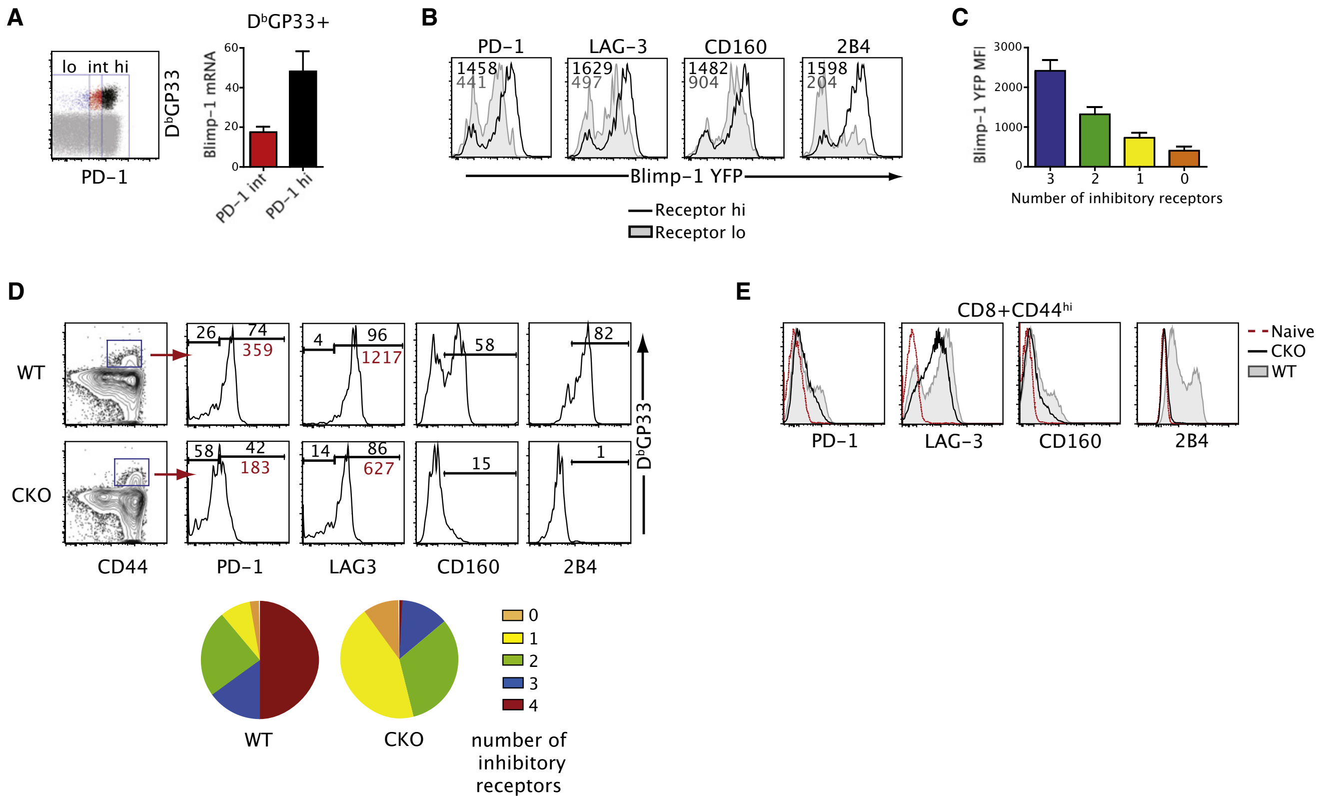

Figure 3. Blimp-1 Expression Correlated with Expression of Inhibitory Receptors on Antigen-Specific CD8+ T Cells during Chronic Viral Infection

(A) Blimp-1 mRNA in PD-1^{hi} and PD-1^{int} subsets of exhausted CD8$^+$ T cells. Plot is gated on WT CD8$^+$ T cells from spleen at day 30 after LCMV clone 13 infection. For qPCR, PD-1^{hi} and PD-1^{int} DbGP33+ CD8$^+$ T cells were sorted from the spleens of LCMV clone 13-infected WT mice. Graph shows increase in Blimp-1 expression over naive CD44^{lo} CD8$^+$ T cells sorted from LCMV Arm immune mice. Error bars show range of expression in triplicate wells.

(B) Blimp-1 YFP reporter expression in inhibitory receptor hi versus lo subsets of exhausted CD8$^+$ T cells. Blimp-1 YFP reporter mice were infected with LCMV clone 13 and YFP expression was analyzed in the spleen at 30 days p.i. Histograms are gated on DbGP33+ CD8$^+$ T cells that are inhibitory receptor^{lo} (shaded) or inhibitory receptor^{hi} (open). Numbers in gray show YFP MFI of shaded histograms, numbers in black represent YFP MFI of open histograms.

(C) Correlation of Blimp-1 expression and number of inhibitory markers expressed. Blimp-1 YFP reporter mice were infected with LCMV clone 13, and inhibitory receptor expression was determined on DbGP33+ CD8$^+$ T cells from the spleen at day 30 p.i. Boolean gating established the populations that expressed a combination of three, two, one, or no inhibitory markers and Blimp-1 YFP MFI was determined for each subgroup. Graph represents two independent experiments. All error bars represent SEM.

(D) Inhibitory receptor expression on antigen-specific CD8$^+$ T cells from WT and gzmB-cre *Prdm1$^{fl/fl}$* mice. GzmB-cre *Prdm1$^{fl/fl}$* and WT littermates were infected with LCMV clone 13 and analyzed on day 30 p.i. for inhibitory marker expression in the spleen. Plots are gated on total CD8$^+$ T cells. Histograms are gated on DbGP33+ CD8$^+$ T cells. Numbers above the gates indicate the percentage of DbGP33+ CD8$^+$ T cells in each gate. Numbers in red are the MFI of each marker. Pie charts represent DbGP33+ CD8$^+$ T cell populations from WT and gzmB-cre *Prdm1$^{fl/fl}$* mice. Each colored slice indicates the fraction of DbGP33+ CD8$^+$ T cells that expresses a combination of four, three, two, one, or no inhibitory receptors.

(E) Inhibitory receptor expression in total CD44^{hi} CD8$^+$ T cells in WT and gzmB-cre *Prdm1$^{fl/fl}$* mice 30 days p.i. Histograms are gated on CD44^{hi} CD8$^+$ T cells from WT (shaded) or gzmB-cre *Prdm1$^{fl/fl}$* (open) mice. Open red histograms represent CD44^{lo} CD8$^+$ T cells from WT mice.

Conditional Blimp-1 Deletion Reduces Expression of Inhibitory Receptors

We next examined whether there was any relationship between the expression of inhibitory receptors and Blimp-1 during chronic LCMV infection. We have previously found that two subsets of exhausted CD8$^+$ T cells can be identified during chronic LCMV infection. One subset expresses intermediate amounts of PD-1 (PD-1^{int}) and is capable of "revival" upon PD-1 pathway blockade, whereas the other expresses high amounts of PD-1 (PD-1^{hi}) and is more terminally differentiated (Blackburn et al., 2008); similar observations have been made during HCV infection in humans (Nakamoto et al., 2008). We sorted virus-specific exhausted PD-1^{int} and PD-1^{hi} CD8$^+$ T cell subsets from chronically infected mice and assessed Blimp-1 mRNA expression by qRT-PCR (Figure 3A). The PD-1^{hi} subset had 2- to 3-fold greater amounts of Blimp-1 mRNA than the PD-1^{int} subset, suggesting a potential role for Blimp-1 in the regulation of PD-1 expression, CD8$^+$ T cell exhaustion, and/or terminal differentiation (Figure 3A).

To examine the relationship between Blimp-1 and inhibitory receptor expression in more detail, we infected Blimp-1 YFP mice with LCMV clone 13 and on day 30 p.i., we examined DbGP33 tetramer+ CD8$^+$ T cells. Blimp-1-driven YFP expression in DbGP33+ CD8$^+$ T cells correlated with PD-1 expression in

multiple tissues, but not with other markers such as CD127 (Figure S3). Next, we gated on exhausted DbGP33+ CD8$^+$ T cells that expressed high versus intermediate or low expression of PD-1, LAG-3, 2B4, or CD160 (Figure 3B). The inhibitory receptor^{hi} subsets consistently had a higher Blimp-1 YFP MFI than the inhibitory receptor$^{int/lo}$ subsets regardless of which inhibitory receptor was examined (Figure 3B). This difference was greatest for 2B4, with a 7.8-fold difference in Blimp-1 YFP MFI in 2B4^{hi} versus 2B4^{lo} exhausted CD8$^+$ T cells. There was also a robust difference in Blimp-1 YFP expression in PD-1^{hi} versus PD-1^{lo} (3.3-fold), LAG-3^{hi} versus LAG-3^{lo} (3.3-fold), and CD160^{hi} versus CD160^{lo} (1.6-fold). Furthermore, using multiparameter flow cytometry to examine coexpression of three inhibitory receptors (PD-1, 2B4, and LAG-3), we found that higher Blimp-1 expression corresponded to an increase in the number of inhibitory receptors coexpressed by the same cell (Figure 3C).

Given the correlation between Blimp-1 YFP expression and inhibitory receptor upregulation, we next examined inhibitory receptor expression in Blimp-1-conditional-deficient mice during chronic LCMV infection. On day 30 p.i., DbGP33-specific CD8$^+$ T cells from gzmB-cre *Prdm1*$^{fl/fl}$ mice expressed considerably less PD-1 and LAG-3 and little CD160 or 2B4 compared to DbGP33+ CD8$^+$ T cells from WT mice (Figure 3D). DbNP396-specific CD8$^+$ T cells also exhibited a decrease in PD-1 expression in gzmB-cre *Prdm1*$^{fl/fl}$ mice as compared to WT mice (Figure S2C). In addition, although nearly half of the DbGP33-specific CD8$^+$ T cells from WT mice coexpressed all four inhibitory receptors, only a small fraction of conditionally Blimp-1-deficient CD8$^+$ T cells expressed all four of these molecules simultaneously and more than half of the gzmB-cre *Prdm1*$^{fl/fl}$ cells expressed zero or only one inhibitory receptor (Figure 3D). This difference in expression of multiple inhibitory receptors in the gzmB-cre *Prdm1*$^{fl/fl}$ mice was not restricted to the DbGP33-specific CD8$^+$ T cell population. The overall population of CD44^{hi} CD8$^+$ T cells from chronically infected WT mice, which contains other LCMV-specific CD8$^+$ T cell populations, had higher expression of PD-1, LAG-3, 2B4, and CD160 compared to CD44^{hi} CD8$^+$ T cells from Blimp-1-conditional-deficient mice (Figure 3D). Together, these data suggest an important role for Blimp-1 in regulating the elevated expression and coexpression pattern of inhibitory receptors on exhausted CD8$^+$ T cells.

Conditional Heterozygous Mice Control Infection More Rapidly than Wild-Type or gzmB-cre *Prdm1*$^{fl/fl}$ Mice

The decrease in inhibitory receptor expression might be expected to improve viral control in the gzmB-cre *Prdm1*$^{fl/fl}$ mice. However, Blimp-1 is known to regulate expression of granzyme B (Gong and Malek, 2007) and is involved in generating terminally differentiated (but functional) effector CD8$^+$ cells after acute infection (Rutishauser et al., 2009; Kallies et al., 2009 [both in this issue of *Immunity*]). Whereas the WT mice controlled viremia by ~day 60 p.i., gzmB-cre *Prdm1*$^{fl/fl}$ mice continued to have high viral titers in the serum 2 months p.i. despite reduced inhibitory receptor expression (Figure 4A). In addition to WT and gzmB-cre *Prdm1*$^{fl/fl}$ mice, we also infected mice with only one intact copy of the Blimp-1 gene (*Prdm1*$^{fl/+}$ × gzmB-cre; conditional heterozygous mice). Conditional haploinsufficiency resulted in mice that controlled viremia more rapidly than gzmB-cre *Prdm1*$^{fl/fl}$ mice (Figure 4A). Unexpectedly, these conditional heterozygous mice also controlled viremia more quickly than the WT mice and had a lower viral load in some tissues than either gzmB-cre *Prdm1*$^{fl/fl}$ or WT mice (Figure 4A). We next tested whether deletion of only one copy of *Prdm1* was sufficient to impact expression of inhibitory receptors. Indeed, these conditional heterozygous mice, like the gzmB-cre *Prdm1*$^{fl/fl}$ mice, had significantly lower MFI of PD-1 compared to virus-specific CD8$^+$ T cells from the WT mice at day 15 p.i., a time point when viral load was similar in all three sets of mice (Figures 4B and 4C). Thus, although mice completely deficient in Blimp-1 had poor long-term viral control compared to WT mice, conditional heterozygous mice had lower PD-1 expression and controlled chronic infection more rapidly than WT mice.

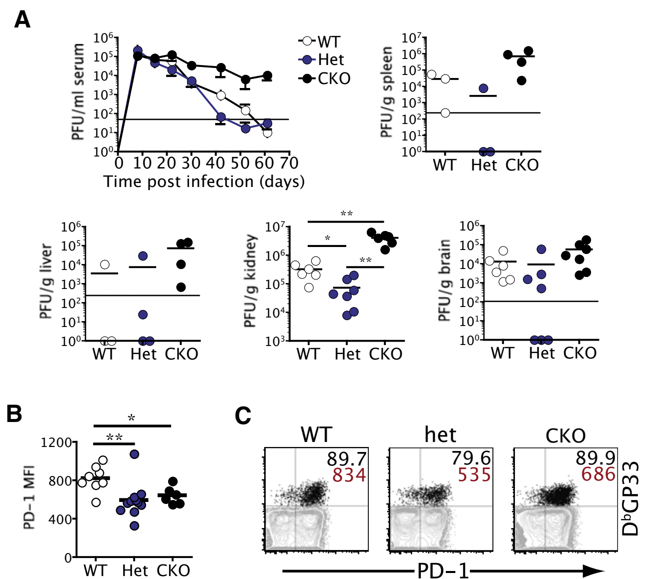

Figure 4. Blimp-1 Haploinsufficiency in Antigen-Specific CD8$^+$ T Cells Leads to Rapid Viral Control

(A) Viral titers in gzmB-cre *Prdm1*$^{fl/fl}$, conditional heterozygous (het), and WT littermates day 60 after LCMV clone 13 infection. Viral load was determined in the serum on the indicated days and in the tissues on day 60 p.i. by plaque assay. Lines on the graphs represent limit of detection. Viral titers are representative of three independent experiments, n = 3–17. For kidney titers, WT versus conditional heterozygous, *p = 0.03; WT versus gzmB-cre *Prdm1*$^{fl/fl}$, **p = 0.003; conditional heterozygous versus CKO, **p = 0.002 by Student's t test. All error bars represent SEM.

(B) PD-1 expression on antigen-specific CD8$^+$ T cells from gzmB-cre *Prdm1*$^{fl/fl}$, conditional heterozygous, and WT mice. PD-1 MFI was measured on DbGP33+ CD8$^+$ T cells from the blood at day 15 p.i. Data are representative of three experiments. WT versus conditional heterozygous, **p = 0.007; WT versus gzmB-cre *Prdm1*$^{fl/fl}$, *p = 0.01 by Student's t test.

(C) Representative plots of PD-1 expression at day 15 p.i. in gzmB-cre *Prdm1*$^{fl/fl}$, conditional heterozygous, and WT mice. Plots are gated on total CD8$^+$ T cells from the blood. Numbers in red indicate the PD-1 MFI of the DbGP33+ CD8$^+$ T cells. Numbers in black are the percent PD-1$^+$ of the tetramer+ cells.

Conditional Blimp-1 Haploinsufficiency Leads to Better Cytotoxicity than Full Conditional Deficiency

We next examined the functional properties of virus-specific CD8$^+$ T cells from WT, conditional heterozygous, and gzmB-cre *Prdm1*$^{fl/fl}$ mice. The ability of antigen-specific CD8$^+$ T cells

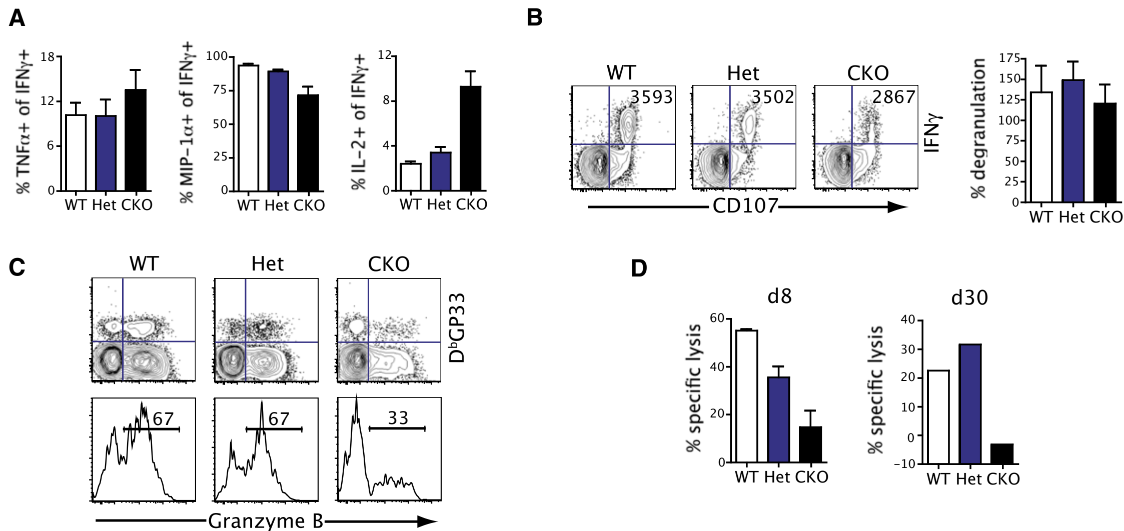

Figure 5. Blimp-1 Regulates Cytoxicity in Antigen-Specific CD8+ T Cells during Chronic Viral Infection

GzmB-cre *Prdm1*$^{fl/fl}$, conditional heterozygous, and WT littermates were infected with LCMV clone 13 and analyzed at day 30 p.i. unless otherwise noted.

(A) Function of CD8+ T cells from gzmB-cre *Prdm1*$^{fl/fl}$, conditional heterozygous, and WT mice. Splenocytes were stimulated with GP33 peptide for 5 hr and the percentage of GP33-specific IFN-γ+ CD8+ T cells that also produced the second indicated cytokine was determined by ICS. Data are representative of two independent experiments, n = 4–6. Error bars are SEM.

(B) Splenocytes from WT, conditional heterozygous, and gzmB-cre *Prdm1*$^{fl/fl}$ mice were stimulated for 5 hr with GP33 peptide, and degranulation based on CD107 staining was determined. Plots are gated on total CD8+ T cells. Numbers in plots indicate MFI of IFN-γ. Graph shows the number of DbGP33+ CD8+ T cells that are also CD107+. Data represent two independent experiments. Error bars are SEM.

(C) Granzyme B expression in CD8+ T cells from gzmB-cre *Prdm1*$^{fl/fl}$, conditional heterozygous, or WT mice. Dot plots are gated on total CD8+ T cells from the spleen, and histograms are gated on DbGP33+ CD8+ T cells. Numbers above the gates indicate the percentage of DbGP33+ CD8+ T cells that express granzyme B.

(D) Specific killing by CD8+ T cells from gzmB-cre *Prdm1*$^{fl/fl}$, heterozygous, and WT mice as determined by an in vitro killing assay on day 8 and day 30 p.i. with LCMV clone 13. Equal numbers of DbGP33+ CD8+ T cells were plated with GP33-peptide-labeled target cells at a 2:1 ratio. After 16–20 hr, specific lysis was calculated. Error bars are SEM.

from the conditional heterozygous mice to coproduce IFN-γ and TNF was not dramatically different from WT or gzmB-cre *Prdm1*$^{fl/fl}$ cells, although the MFI of IFN-γ was higher in WT and conditional heterozygous CD8+ T cells compared to gzmB-cre *Prdm1*$^{fl/fl}$ cells (Figures 5A and 5B). Coproduction of IFN-γ and MIP-1α was slightly increased in conditional heterozygous compared to gzmB-cre *Prdm1*$^{fl/fl}$ CD8+ T cells, but conditional heterozygous and WT CD8+ T cells were similar in this regard (Figure 5A). IL-2 production was high only in the gzmB-cre *Prdm1*$^{fl/fl}$ mice, suggesting that even one copy of *Prdm1* was sufficient to repress IL-2 during chronic infection (Figure 5A). Thus, production of antiviral cytokines by exhausted CD8+ T cells was similar regardless of the number of copies of *Prdm1*.

Blimp-1 expression could be particularly relevant for cytotoxic activity given that Blimp-1 has been implicated in the expression of granzyme B (Gong and Malek, 2007). Virus-specific CD8+ T cells from WT, conditional heterozygous, and gzmB-cre *Prdm1*$^{fl/fl}$ mice were equally capable of degranulating on the basis of CD107a surface expression after peptide stimulation (Figure 5B). However, antigen-specific CD8+ T cells from gzmB-cre *Prdm1*$^{fl/fl}$ mice were severely deficient in expression of granzyme B compared to both conditional heterozygous and WT mice (Figure 5C). The ability of antigen-specific CD8+ T cells from conditionally Blimp-1-deficient mice to lyse peptide-coated targets was also substantially impaired by day 8 p.i. and was essentially absent by day 30 p.i., although killing was similar by CD8+ T cells from the conditional heterozygous and WT mice (Figure 5D). Normally, during chronic LCMV infection, cytotoxicity declines as the infection progresses (Wherry et al., 2003). Indeed, the degree of killing by WT and gzmB-cre *Prdm1*$^{fl/fl}$ cells decreased by day 30, but this residual killing was still higher than that observed in the complete absence of Blimp-1 (Figure 5D). In contrast, LCMV Arm immune mice sustain the ability to robustly reactivate killing activity (Figure S4) (Barber et al., 2003). These observations suggest that although high expression of Blimp-1 was detrimental to CD8+ T cell responses and was associated with substantial upregulation of inhibitory receptors, retention of one intact copy of *Prdm1* and likely intermediate expression of Blimp-1 was required to induce and/or maintain cytotoxic potential. Thus, conditional Blimp-1 deficiency indicated a key role for this transcription factor in controlling some aspects of T cell exhaustion (increased inhibitory receptors, repression of memory T cell differentiation), but complete loss of Blimp-1 also compromised the low cytotoxic activity found in WT or conditional Blimp-1 heterozygous CD8+ T cells during chronic LCMV infection.

The Role of Blimp-1 in CD8+ T Cell Exhaustion Is Cell Intrinsic

Blimp-1 appears to be a good candidate for transcriptional control of some aspects of CD8+ T cell exhaustion during chronic viral infection. The data described above, however, do not rule

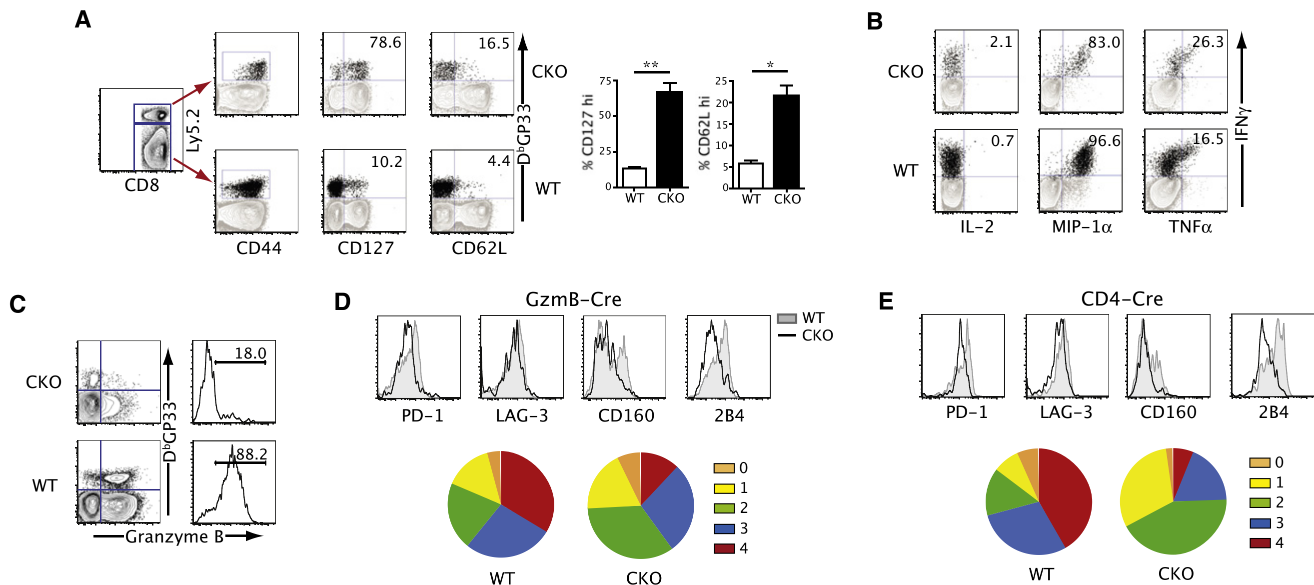

Figure 6. The Role of Blimp-1 in CD8+ T Cells during Chronic LCMV Infection Was Cell Intrinsic

(A) Phenotype of CD8+ T cells from mixed BM chimeras. Left-most plot is gated on total CD8+ T cells from the spleen. The top row of plots is gated on Ly5.2+ CD8+ T cells (gzmB-cre *Prdm1^fl/fl^*), and the bottom row is gated on Ly5.2- CD8+ T cells (WT). Numbers in plots indicate the percentage of DbGP33+ CD8+ T cells positive for each marker. Numbers are graphed to the right. Data are representative of two experiments, n = 4. Error bars are SEM. For CD127, **p = 0.005 and for CD62L, *p = 0.01 by Student's t test.

(B) Effector function of CD8+ T cells from mixed BM chimeras. Splenocytes were stimulated with GP33 peptide for 5 hr. The top row of plots is gated on gzmB-cre *Prdm1^fl/fl^*, and the bottom row is gated on WT CD8+ T cells. Numbers in plots indicate the percentage of IFN-γ+ CD8+ T cells that also produce a second cytokine.

(C) Granzyme B expression in CD8+ T cells from mixed BM chimeras. Plots are gated on CD8+ T cells from the spleen. Histograms are gated on DbGP33+ CD8+ T cells. The top row is gated on gzmB-cre *Prdm1^fl/fl^*, and bottom row is gated on WT. Numbers above gates show percentage of DbGP33+ CD8+ T cells that express granzyme B.

(D) Inhibitory receptor expression on CD8+ T cells from mixed BM chimeras. Histograms are gated on WT DbGP33+ CD8+ T cells (shaded) or gzmB-cre *Prdm1^fl/fl^* DbGP33+ CD8+ T cells (open). Pie charts show either WT or gzmB-cre *Prdm1^fl/fl^* DbGP33+ CD8+ T cells. Colored slices indicate the fraction of the total population that expressed a combination of four, three, two, one, or no inhibitory markers.

(E) Inhibitory receptor expression on CD8+ T cells from mixed BM chimeras reconstituted with CD4-cre *Prdm1^fl/fl^* and WT bone marrow. Histograms are gated on WT DbGP33+ CD8+ T cells (shaded) or open CD4-cre *Prdm1^fl/fl^* DbGP33+ CD8+ T cells (open). Pie charts show either WT or CD4-cre *Prdm1^fl/fl^* DbGP33+ CD8+ T cells.

out the possibility that Blimp-1 deficiency in other cell types, or changes in environmental factors such viral load and/or pathogenesis of infection, could have an impact on antigen-specific CD8+ T cells. To examine the cell-intrinsic role of Blimp-1 in CD8+ T cell exhaustion, we generated mixed bone marrow (BM) chimeras by injecting equal numbers of T and B cell-depleted bone marrow cells from Ly5.2+ gzmB-cre *Prdm1^fl/fl^* and Ly5.1+ WT animals into lethally irradiated Ly5.1+ recipient mice (Figure S5). After reconstitution, the mixed BM chimeras were infected with LCMV clone 13 and analyzed 4 weeks after infection (Figure S5).

At 1 month after infection, the differentiation state and function of virus-specific WT and gzmB-cre *Prdm1^fl/fl^* CD8+ T cells from viremic (data not shown) mixed BM chimeras was examined. As observed in separate WT and gzmB-cre *Prdm1^fl/fl^* mice, a higher proportion of Blimp-1-deficient antigen-specific CD8+ T cells in the mixed chimeras expressed CD127 and CD62L compared to the WT antigen-specific CD8+ T cells (Figure 6A). In addition, the functional profiles of WT and gzmB-cre *Prdm1^fl/fl^* cells in the mixed chimeras paralleled observations in individual WT and gzmB-cre *Prdm1^fl/fl^* mice. Production of IL-2 and TNF were elevated, and MIP-1α was slightly decreased in gzmB-cre *Prdm1^fl/fl^* CD8+ T cells compared to WT CD8+ T cells (Figure 6B). In the mixed BM chimeras, the virus-specific gzmB-cre *Prdm1^fl/fl^* cells also had defective granzyme B expression, whereas the WT cells sustained expression of this protein (Figure 6C). Finally, gzmB-cre *Prdm1^fl/fl^* antigen-specific CD8+ T cells in the mixed BM chimeras expressed lower amounts of inhibitory receptors compared to WT antigen-specific CD8+ T cells in the same animals (Figure 6D). Although we did not observe as drastic a difference in LAG-3 expression in the mixed chimeras as we did in individual mice, PD-1 expression was decreased in Blimp-1-deficient antigen-specific CD8+ T cells compared to WT antigen-specific CD8+ T cells in the same host (Figure 6D). The CD160^hi^ and 2B4^hi^ subsets present in WT virus-specific CD8+ T cells were also dramatically reduced or absent from the gzmB-cre *Prdm1^fl/fl^* antigen-specific CD8+ T cell populations (Figure 6D). The pattern of coexpression of multiple inhibitory markers was also clearly distinct for gzmB-cre *Prdm1^fl/fl^* and WT DbGP33-specific CD8+ T cells in the mixed chimeras. Whereas less than half of the gzmB-cre *Prdm1^fl/fl^* DbGP33-specific CD8+ T cells coexpressed three or more inhibitory receptors, nearly two-thirds of the WT cells in the same mice coexpressed three or more inhibitory receptors (Figure 6D). In parallel experiments, we also generated mixed chimeras by using BM from WT and *Prdm1^fl/fl^* X CD4-cre mice and observed

a similar impact on the pattern of memory T cell differentiation, function (data not shown), and expression of inhibitory receptors (Figure 6E). This difference in inhibitory receptor expression between the CD4-cre *Prdm1*$^{fl/fl}$ CD8$^+$ T cells and WT CD8$^+$ T cells was apparent in both the expression level of individual inhibitory markers and the coexpression patterns (Figure 6E). These observations suggest that the changes observed in the gzmB-cre *Prdm1*$^{fl/fl}$ CD8$^+$ T cells were not due to the timing of cre-mediated recombination or deletion of Blimp-1 in granzyme B-expressing non-CD8$^+$ T cells. Thus, the mixed BM chimeras indicate that Blimp-1 has a cell-intrinsic role in regulating inhibitory receptor expression and identify Blimp-1 as a transcriptional regulator of CD8$^+$ T cell exhaustion during chronic viral infections.

DISCUSSION

Although several transcription factors have been shown to regulate effector and memory T cell differentiation after acute infections, the transcriptional mechanisms of T cell exhaustion have been unclear. In this study, we identify a role for the transcriptional repressor Blimp-1 in regulating several defining features of CD8$^+$ T cell exhaustion during chronic viral infection. Exhausted CD8$^+$ T cells had substantially higher expression of Blimp-1 than functional effector or memory CD8$^+$ T cells generated after acute infection. Higher expression of Blimp-1 correlated with upregulated inhibitory receptor expression and repression of memory T cell properties. Conversely, conditional deletion of Blimp-1 resulted in reduced inhibitory receptor expression by exhausted CD8$^+$ T cells and demonstrated the central involvement of Blimp-1 in preventing normal memory T cell differentiation during chronic viral infection. These studies, however, also indicated that although high expression of Blimp-1 was associated with key aspects of T cell exhaustion, some Blimp-1 was essential for sustained T cell function including cytolysis, which in turn was necessary for eventual control of chronic infection. Thus, Blimp-1 appears to act as a transcriptional rheostat controlling CD8$^+$ T cell functionality at low amounts and CD8$^+$ T cell exhaustion when highly expressed.

CD8$^+$ T cell exhaustion is a common feature of many chronic viral infections in both animal models as well as humans and is a likely reason for poor pathogen control in these situations (Shin and Wherry, 2007). For example, T cell dysfunction, including expression of inhibitory receptors, occurs not only in mice but also during infections such as SIV in monkeys and HIV, HBV, and HCV in humans (Shin and Wherry, 2007). Thus, there has been considerable interest in the molecular mechanisms of T cell exhaustion. High and sustained expression of PD-1 has emerged as a hallmark of T cell exhaustion, and blocking this pathway can reinvigorate immune responses during persisting infections (Freeman et al., 2006; Sharpe et al., 2007). In the current study, Blimp-1 expression was two to three times higher in the more terminally differentiated PD-1^{hi} subset of exhausted CD8$^+$ T cells compared to the PD-1$^{int\text{-}lo}$ subset that can be "revived" by antibody blockade (Blackburn et al., 2008; Nakamoto et al., 2008). Other inhibitory receptors, including LAG-3, 2B4, and CD160, are also upregulated by exhausted CD8$^+$ T cells, and these pathways cooperate to negatively regulate CD8$^+$ T cell responses during chronic viral infection (Blackburn et al., 2009). Blimp-1 also regulated expression of these additional inhibitory receptors, given that in the absence of Blimp-1, the expression of these receptors was lower during chronic LCMV infection. It should be noted inhibitory receptors are unlikely to be the only regulatory pathways involved in T cell exhaustion. Key roles for elevated IL-10 and the loss of IL-21 have been described (Brooks et al., 2006; Ejrnaes et al., 2006; Elsaesser et al., 2009; Frohlich et al., 2009; Yi et al., 2009), and CD8$^+$ T cell exhaustion is associated with dramatic changes in global gene expression (Wherry et al., 2007). It is interesting that despite lower expression of inhibitory receptors in the absence of Blimp-1, these CD8$^+$ T cells remained poor cytokine producers. It is possible that other negative regulatory pathways compensate in this setting or that Blimp-1 positively regulates antiviral cytokine expression. A third possibility is that Blimp-1 controls one "module" of the transcriptional program of T cell exhaustion including inhibitory receptor expression and memory repression. Although inhibitory receptors are clearly linked to T cell function, another layer of transcriptional control that influences expression of antiviral cytokines could exist. Such an idea of overlapping transcriptional modules is emerging for Foxp3$^+$ Treg cells (Koch et al., 2009; Zheng et al., 2009). Future studies are necessary to dissect additional transcriptional pathways associated with T cell exhaustion and Blimp-1-independent regulation of cytokine production.

After activation during an acute infection, antigen-specific effector CD8$^+$ T cells can adopt one of two fates: terminally differentiated effector CD8$^+$ T cells or memory CD8$^+$ T cell precursors (Chang et al., 2007; Joshi et al., 2007; Sarkar et al., 2008). The transcription factor Tbet has been implicated in the development of terminally differentiated effector CD8$^+$ T cells versus memory precursors (Intlekofer et al., 2007; Joshi et al., 2007; Sarkar et al., 2008), and other transcription factors such as Eomesodermin (Intlekofer et al., 2005), Id2 (Cannarile et al., 2006), Bcl6, and Bcl6b (Ichii et al., 2004; Manders et al., 2005), and Bmi1 (Heffner and Fearon, 2007) can also regulate memory CD8$^+$ T cell differentiation after acute infection. During chronic infection, antigen-specific CD8$^+$ T cells differentiate into a population transcriptionally distinct from effector and memory CD8$^+$ T cells present after acute infection (Wherry et al., 2007), but the transcription factors involved have been unclear. In the germinal center, Blimp-1 controls the terminal differentiation of B cells into long-lived plasma cells and also represses the development of memory B cells (Calame, 2006) and Blimp-1 has a key role in determining cell-fate decisions in many settings outside the immune system (Hayashi et al., 2007; Ohinata et al., 2005; Horsley et al., 2006; Roy and Ng, 2004; de Souza et al., 1999). During chronic LCMV infection, CD8$^+$ T cell exhaustion becomes established progressively after the effector phase and important events in this progression, such as the appearance of the PD-1^{hi} subset of exhausted CD8$^+$ T cells (Barber et al., 2006; Wherry et al., 2007; Blackburn et al., 2008), correspond to the further upregulation of Blimp-1 observed 2–4 weeks p.i. In addition, because mice with one intact copy of the *Prdm1* gene were not intermediate between WT and gzmB-cre *Prdm1*$^{fl/fl}$ mice but rather achieved more efficient control of infection than WT or gzmB-cre *Prdm1*$^{fl/fl}$ mice, it is likely that the amount of Blimp-1 has a crucial impact on CD8$^+$ T cell differentiation and exhaustion during chronic viral infection. It appeared that low or intermediate expression of Blimp-1 was required for some

effector functions, whereas the high expression of Blimp-1 that occurred during chronic viral infection promoted CD8+ T cell exhaustion and repressed memory differentiation. Thus, our data is consistent with the idea that the considerable upregulation of Blimp-1 in virus-specific CD8+ T cells by ~1 month of chronic viral infection is a developmental switch that promotes key aspects of CD8+ T cell exhaustion.

Our studies also point to a critical role for sustained cytolytic potential during chronic viral infections. Recent work has demonstrated the importance of cytotoxicity in long-term control of chronic infections in humans (Appay et al., 2000; Migueles et al., 2002; Migueles et al., 2008; Trabattoni et al., 2004). Although exhausted CD8+ T cells are known to have partial defects in killing compared to highly functional effector or memory CD8+ T cells (Wherry et al., 2003), some residual cytotoxicity by antigen-specific CD8+ T cells can be maintained in vivo (Agnellini et al., 2007). Blimp-1 conditional heterozygous mice controlled virus substantially more quickly than GzmB-cre *Prdm1*$^{fl/fl}$ mice, and this difference corresponded to sustained cytolysis in the conditional heterozygous mice compared to the GzmB-cre *Prdm1*$^{fl/fl}$ mice. However, conditional heterozygous mice also controlled virus in vivo more rapidly than WT mice, despite similar killing and cytokine production in vitro. It is possible that lower PD-1 expression by conditional heterozygous mice led to improvements in effector function in vivo that were not obvious in vitro, as has been observed with blockade of other inhibitory pathways such as LAG-3 (Blackburn et al., 2009). Conditional heterozygous mice also had slightly more virus-specific CD8+ T cells than WT mice. Higher numbers of virus-specific CD8+ T cells and improved effector functions in vivo by conditional heterozygous mice could account for this more efficient control of infection compared to WT mice, but future studies are necessary to investigate these issues further.

In summary, these studies provide a framework to begin dissecting Blimp-1 targets, regulation of Blimp-1 activity, and other transcriptional pathways involved in T cell dysfunction during chronic infection. Although clearly additional transcription factors and pathways contribute to T cell exhaustion, our results identify Blimp-1 as a transcriptional regulator of CD8+ T cell exhaustion and repressor of memory CD8+ T cell differentiation during chronic viral infection.

EXPERIMENTAL PROCEDURES

Animals and Viruses

Four- to six-week-old C57BL/6 or C57BL/6 Ly5.2CR (Ly5.1+) mice were purchased from NCI. Rosa26-f/stop/f-YFP mice were purchased from Jackson Laboratories. BAC transgenic Blimp-1 YFP reporter mice were from E. Meffre (Yale University, New Haven, CT). *Prdm1*$^{fl/fl}$ mice were from K. Calame (Columbia University, New York, NY), granzyme B-cre mice were from J. Jacob (Emory University, Atlanta, GA), and CD4-cre mice were from S. Reiner (University of Pennsylvania, Philadelphia, PA). Mouse strains were crossed and mice were bred, maintained in the Wistar Institute, and used in accordance with IACUC guidelines. Mice were infected with 2×10^5 plaque-forming units (PFUs) of LCMV Armstrong (Arm) i.p. or 2×10^6 PFU LCMV clone-13 (Cl-13) i.v. as described (Wherry et al., 2003). Virus was grown and titered as described (Wherry et al., 2003).

Lymphocyte Isolation and Flow Cytometry

Lymphocyte isolation from lymphoid and nonlymphoid tissues, surface stains, intracellular cytokine stains (ICS), and CD107 assay were performed as previously described (Barber et al., 2006; Wherry et al., 2003). All antibodies were purchased from Biolegend except for CD127, CD160, TNF, IL-2 (eBioscience), 2B4 (eBioscience, BD Biosciences), LAG-3 (AbD Serotec), granzyme B (Caltag), and MIP-1α (R&D Systems). LIVE/DEAD dead-cell stain, CFSE, streptavidin-APC, and streptavidin-Quantum dot 655 were purchased from Invitrogen. MHC class I peptide tetramers were made and used as described previously (Wherry et al., 2004; Wherry et al., 2003). All flow cytometry data were acquired on an LSRII (BD Biosciences) and analyzed by FlowJo (Treestar). Pie charts were created with the Pestle and SPICE programs (Mario Roederer; Vaccine Research Center, NIAID, NIH).

Bone Marrow Chimeras

Bone marrow cells from Ly5.1+ WT and gzmB-cre *Prdm1*$^{fl/fl}$ (either *Prdm1*$^{fl/fl}$ × gzmB-cre or *Prdm1*$^{fl/fl}$ × CD4-cre) mice were T and B cell depleted with MACS magnetic beads (Miltenyi Biotec) and adoptively transferred at a 1:1 ratio into lethally irradiated (1000 rad) C57BL/6 Ly5.2Cr (Ly5.1+) mice. Mice were fed antibiotic for 2 weeks and allowed to reconstitute for eight weeks before use.

Quantitative PCR

Cells were sorted on a FACSAria (BD Biosciences). RNA extraction was performed with Trizol (Invitrogen). cDNA was generated using the High Capacity cDNA Archive Kit (Applied Biosystems). Relative quantification real-time PCR was performed on an ABI Prism 7000 with primers purchased from Applied Biosystems. HPRT was used as an endogenous control, and gene expression measured as increase in expression over naive CD8+ T cells.

In Vitro Killing Assays

CD8+ T cells were purified with MACS magnetic beads (Miltenyi Biotec) from spleens of gzmB-cre *Prdm1*$^{f/f}$, heterozygous, and WT mice in accordance with the manufacturer's protocol. Target cells were either labeled with GP33 peptide or SIINFEKL peptide and CFSE labeled at two different concentrations. Equal numbers of DbGP33+ CD8+ T cells were plated at a 2:1 ratio with the labeled target cells, and total cell numbers were normalized with naive splenocytes. The cells were incubated at 37°C for 16–20 hr and specific lysis was calculated as described (Hermans et al., 2004).

SUPPLEMENTAL DATA

Supplemental Data include five figures and can be found with this article online at http://www.cell.com/immunity/supplemental/S1074-7613(09)00319-7.

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