animals could still be cross-presented to $CD8^+$ T cells (Bertholet et al., 2006). Because TAP is required for the translocation of peptides from the cytoplasm to the ER lumen, these results suggest that direct loading of exogenous peptides on MHC class I molecules occurred directly in the phagosome lumen. The efficiency of this process appeared, however, to be limited. Hence, Irgm3 would enhance cross-presentation by improving the molecular mechanisms connecting the early step of protein processing in phagosomes with the loading of peptides on MHC class I molecules. A surprising result revealed by Bougnères et al. (2009) is that although $Irgm3^+$ LBs were often observed in the close vicinity of phagosomes, no noticeable accumulation of Irgm3 was observed on these organelles by immunofluorescence. This observation suggests that Irgm3 has an indirect effect on the phagosomal cross-presentation machinery, possibly through LBs. We have shown recently, by using quantitative proteomics analyses, that the relative amount of several members of the p47 family, including Irgm3, increased markedly on phagosomes isolated from macrophages treated with IFN-γ, compared to control cells (Trost et al., 2009). These results would rather argue that Irgm3 is directly involved in the ability of phagosomes to participate in cross-presentation. Another effect of IFN-γ highlighted in recent studies is the ability of this cytokine to modulate the conditions encountered in the phagosome lumen to promote a limited hydrolysis of proteins favoring the generation of peptides suited for loading on MHC molecules (Yates et al., 2007; Trost et al., 2009). Interestingly, Bougnères et al. (2009) indicate that Irgm3 inhibits phagosome maturation, without altering the rate of phagocytosis, a process that should, in principle, limit the rapid proteolysis of peptides and favor antigen presentation.

More studies will highlight the relative contribution of Irgm3 and LBs to cross-presentation, as well as the role of this protein in the interaction between phagosomes and LBs. A thorough characterization of the molecular organization and biogenesis of LBs will also provide key elements to understanding the molecular nature of the interaction occurring between LBs and the other organelles involved in cross-presentation, including phagosomes and autophagosomes. Considering the fact that LBs display a monolayer membrane, one might argue that this type of interaction might influence the lipid organization of the outer membrane of these organelles and/or the association of cytoplasmic anchored proteins like Irgm3.

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Blimp Hovers over T Cell Immunity

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The functions of T lymphocytes are regulated by transcription factors controlling gene expression. Three studies in this issue of *Immunity* (Kallies et al., 2009; Rutishauser et al., 2009; Shin et al., 2009) indicate that the transcriptional repressor Blimp-1 promotes the development of short-lived effector cells and regulates clonal exhaustion.

The B cell transcriptional repressor Blimp-1 (B lymphocyte-induced maturation protein-1), encoded by the *Prdm1* gene locus, is known as a decision maker in the fate of B cells by regulating genes promoting B cell terminal differentiation into plasma cells but not into memory B cells (Shaffer et al., 2002; Shapiro-Shelef et al., 2003). More recent studies, including three in this issue of *Immunity*, indicate that Blimp-1 may provide similar functions for $CD8^+$ T cells, by promoting the terminal differentiation of most into short-lived cytotoxic T lymphocytes (CTLs) rather than long-lived central memory (CM) T cells (Kallies et al., 2009; Rutishauser et al., 2009; Shin et al., 2009). Although originally defined in B cells, Blimp-1 is important for the development of multiple lineages during embryogenesis and is also known to promote terminal differentiation of skin keratinocytes (John and Garrett-Sinha, 2009). Mice with T cell-specific deletions in Blimp-1 develop severe

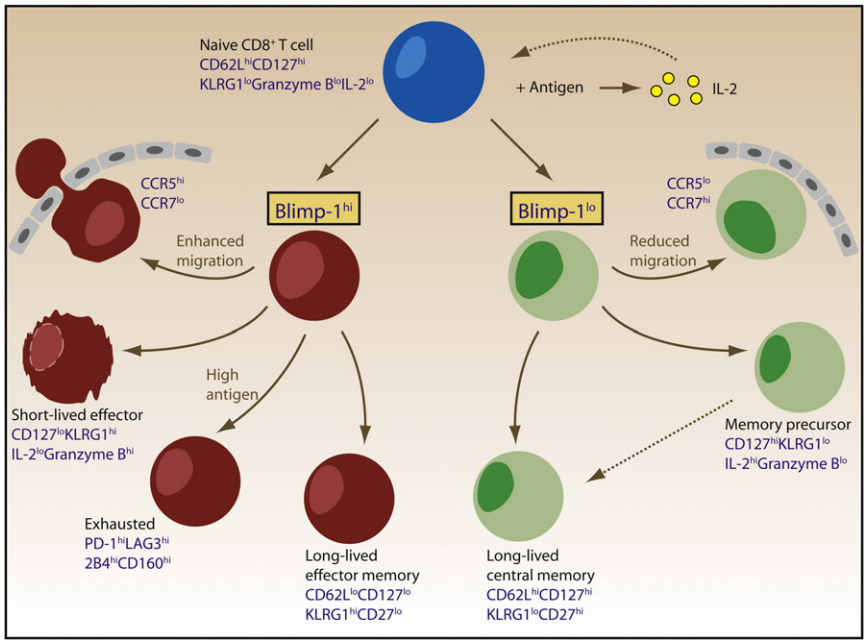

Figure 1. Blimp-1 and the Fates of T Cells during Viral Infections
This figure portrays fates of CD8 T cells that either do or do not express Blimp-1 during a viral infection. Naive T cells sensing antigen will secrete IL-2 and other cytokines and ultimately enter different differentiation pathways. IL-2 can stimulate expression of Blimp-1, which, in turn, will shut down IL-2 expression. Blimp-1$^+$ cells can migrate into peripheral tissues, become short-lived effector cells under conditions of low antigen load, and become clonally exhausted under conditions of high antigen load. Some survive longer term as effector memory cells. Cells lacking Blimp-1 migrate relatively poorly into the periphery, produce IL-2, and are most of the memory cell precursors, evolving to become central memory cells.

immunopathology, indicating a requirement for Blimp-1 in normal T cell homeostasis (Kallies et al., 2006). Blimp-1 is induced in T cells by IL-2 and other cytokines, but its expression represses *Il2* gene transcription (Martins et al., 2008). Thus, it can serve in a negative feedback process, altering T cell gene expression.

How then, does the activity of Blimp-1 act on the T cell response to viral infection? Viruses are notoriously strong stimulators of CD8$^+$ CTLs, which can lyse virus-infected targets and control viral replication by secreting antiviral cytokines such as interferon-γ (IFN-γ). Virus-specific T cells undergo a programmed expansion and differentiation into effector cells and thereafter contract in numbers because of apoptosis, leaving a smaller but stable subpopulation of memory T cells that can vigorously respond to reinfection at a later date (Harty and Badovinac, 2008). T cells activated during the acute infection can express different antigens that predict their fate. For example, mouse memory CD8$^+$ T cells are more likely to be derived from T cells expressing high amounts of the IL-7 receptor (CD127) and low amounts of the natural killer cell receptor KLRG1, whereas short-lived effector cells are more likely to be CD127^{lo}, KLRG1^{hi} (Parish and Kaech, 2009). Overwhelming viral loads, however, can drive T cells either into a clonal deletion by apoptosis or into a functional exhaustion associated with the expression of T cell inhibitory receptors, such as PD-1, LAG-3, 2B4, and CD160 (Blackburn et al., 2009). These new studies indicate that Blimp-1 is an important regulator of all of these fates.

Rutishauser et al. (2009) examined the role of Blimp-1 during the CD8$^+$ T cell response of mice acutely infected with lymphocytic choriomeningitis virus (LCMV), strain Armstrong. Using mice in which the gene for yellow fluorescent protein (*yfp*) was under the control of Blimp-1 promoter elements, they found high expression of Blimp-1 in most cells during the acute response and lower expression in memory cells after resolution of infection. Blimp-1 mRNA, protein, and YFP reporter expression were high in the CD127^{lo}KLRG1^{hi} population of short-lived effector cells and low in the memory precursor CD127^{hi}KLRG1^{lo} cells. In the memory state, Blimp-1 expression remained elevated for some time in effector memory (EM) phenotype cells (CD127^{lo} KLRG1^{hi}CD27^{lo}CD62L^{lo}) but low in the more frequent CM phenotype cells (CD127^{hi}KLRG1^{lo}CD27^{hi}CD62L^{hi}), which are considered highly proliferative on antigenic rechallenge. This suggests that Blimp1 expression distinguishes the effector cell lineage from the CM lineage. These authors crossed mice carrying the loxP-flanked *Pdrm1* gene (encoding Blimp-1) with mice carrying cre-recombinase under control of the Granzyme B promoter, such that T cells would delete the *Prdm1* gene when they became activated during infection. These *Prdm1*$^{-/-}$ mice had reduced frequencies of short-lived effector cells and a reduced contraction phase, coupled with enhanced overall survival of virus-specific CD8$^+$ T cells.

During the acute LCMV infection, Blimp-1 deficiency yielded populations of CD8$^+$ T cells that could produce relatively normal amounts of IFN-γ and TNF, but they produced less Granzyme B, which is involved in cytotoxicity, and much more IL-2 than wild-type (WT) cells. The relatively low IL-2 in Blimp-1$^+$ cells and high amounts in Blimp-1$^-$ cells is consistent with Blimp-1 being a transcriptional repressor of the *Il2* gene (Martins et al., 2008). These same functional properties of *Prdm1*$^{-/-}$ T cells were also noted in the studies of Kallies et al. (2009) and Shin et al. (2009), and all three groups correlated Blimp-1 expression with short-lived effector cells and the absence of Blimp-1 expression with the generation of CM cells (Figure 1).

Shin et al. (2009) compared Blimp1 expression in T cells from mice undergoing acute LCMV-strain Armstrong infection to those from mice undergoing a persistent infection established by high-dose inoculation with the highly disseminating clone 13 variant of LCMV. In both infections, Blimp-1 mRNA or Blimp-1-YFP was elevated initially, but Blimp-1 expression then became especially elevated during the persistent infection. Blimp-1 was most highly expressed in T cells specific for the LCMV-encoded NP396 peptide, and these are usually the first T cells to exhaust or undergo apoptosis during persistent LCMV infection. Cells undergoing clonal exhaustion

during persistent infection express high amounts of PD-1, and the PD-1^{hi} cells expressed 2- to 3-fold more Blimp-1 mRNA than did a subset expressing lower amounts of PD-1. In general, expression of Blimp1-YFP was highest in CD8$^+$ T cells expressing T cell-exhaustion-associated inhibitory receptors, such as PD-1, Lag-3, 2B4, and CD160. LCMV-specific T cells from persistently infected Blimp-1-deficient mice expressed low amounts of inhibitory receptors, resisted clonal exhaustion, and were at higher frequencies than those in infected WT mice, and many expressed high amounts of CD127 and CD62L, consistent with a CM phenotype. Decreased inhibitory receptor expression on T cells in the absence of Blimp-1 might predict that *Prdm1*$^{-/-}$ mice would clear persistent LCMV quickly, but this did not happen. Blimp-1 was also needed for effector cell function, and *Prdm1*$^{-/-}$ T cells had reduced cytotoxicity against viral-peptide-coated targets. However, ''haploinsufficient'' mice, constructed to have only one copy of the *Prdm1* gene, had reduced expression of Blimp-1 and cleared LCMV better, presumably because this intermediate amount of Blimp-1 allowed for effector cell function without upregulating inhibitory receptors (Shin et al., 2009).

Kallies et al. (2009) made chimeric mice with an immune system derived from fetal liver cells of mice whose *Prdm1* gene was inactivated by a green fluorescent protein knock-in (*Prdm1*^{gfp}) and found that those mice suffered severely from an influenza A virus (IAV) infection that was tolerated in control mice. Bone marrow chimeric mice were next made with a combination of *Prdm1*$^{gfp/gfp}$ (Blimp-1-deficient) and *Prdm1*$^{+/+}$ (WT control), and the presence of the WT cells allowed for enhanced resistance to IAV and the opportunity to compare the responses of *Prdm1*$^{gfp/gfp}$ to WT cells under similar conditions of viral load. These Blimp-1-deficient T cells in IAV-challenged chimeric mice lacked the short-lived effector phenotype and highly expressed the memory cell transcription factor eomesodermin and the memory cell-associated transcriptional repressor Bcl-6, also detected in the molecular screens of Rutishauser et al. (2009). After resolution of infection, a high proportion of Blimp-1-deficient cells were CD127^{hi}CD62L^{hi}, consistent with a CM phenotype. Thus, all three studies indicate that Blimp-1 expression is associated with effector cells and its absence is associated with long-lasting CM cells or their precursors.

Kallies et al. (2009) also detected a potentially important cell migration phenotype. After a primary IAV infection of chimeric mice, the Blimp-1-deficient CD8$^+$ T cell frequency was relatively normal in the spleen but markedly reduced in the lungs, in comparison to WT cells. On IAV challenge of mice previously immunized with another IAV strain, the *Prdm1*$^{gfp/gfp}$ T cells were elevated in the spleen but again low in the lung, compared to WT controls. These experiments suggested that Blimp-1 was needed for trafficking into the lung, and indeed, in the Blimp-1-deficient cells the expression of the lung-homing chemokine receptor CCR5 was reduced, and expression of CCR7, important for recruitment into the spleen and lymph nodes, was elevated.

Together, these reports indicate that Blimp-1 promotes the generation of short-lived effector T cells, the generation of clonally exhausted T cells, and the migration of T cells out of the spleen and lymph nodes and into peripheral tissues. Blimp-1 expression does permit the generation of some longer-lived EM cells, but its absence allows for the generation of long-term CM cells, which are thought to have higher proliferative potential on secondary challenge. Blimp-1 is a transcriptional repressor of IL-2 (Martins, et al., 2008), and the generation of polyfunctional T cells having the ability to make IL-2 has been correlated with more protective antiviral responses in vaccine studies (Seder et al., 2008). What remains unclear is why some T cells express Blimp-1 and others do not, but cytokines can stimulate its expression. The contraction phase of T cell responses is less dramatic when T cells are stimulated in low inflammatory environments (Harty and Badovinac, 2008). Perhaps high amounts of inflammatory cytokines promote the expression of Blimp-1 and the generation of short-lived effector cells, whose expansion and then loss would cause a greater contraction.

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