Mixed hematopoietic or T-cell chimerism above a minimal threshold restores perforin-dependent immune regulation in perforin-deficient mice

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Key Points

- Perforin deficiency causes immune dysregulation and hemophagocytic lymphohistiocytosis, which requires allogeneic HCT for long-term cure.
- In prf−/− mice, wild-type hematopoietic or CD8+ T-cell engraftment of only 10-20% is sufficient to reestablish normal immune regulation.

Defects in perforin and related genes lead to a loss of normal immune regulation and underlie hemophagocytic lymphohistiocytosis (HLH), which requires hematopoietic cell transplantation for long-term cure. However, transplantation may be complicated by the development of mixed chimerism and uncertainty regarding the risk of HLH recurrence. To help clarify this risk and investigate how perforin influences immune activation, we studied perforin-mediated immune regulation in the context of mixed chimerism using a murine model of HLH. We found that there is a distinct threshold of ∼10% to 20% perforin expression with either mixed hematopoietic or CD8+ T-cell chimerism, above which immune regulation was reestablished. These findings demonstrate that perforin-mediated immunoregulation functions in trans and are consistent with a feedback model in which cytotoxic T cells control immune activation by killing dendritic cells. These findings also suggest rational targets for maintenance of minimal posttransplant chimerism and for therapeutic strategies involving gene correction. (Blood. 2013;122(15):2618-2621)

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a unique immunoregulatory disorder in which defects in perforin-dependent cytotoxicity lead to a syndrome of fatal immune activation.1,2 Short-term treatment of HLH involves immunosuppressive therapies, while long-term treatment requires allogeneic hematopoietic cell transplantation (HCT) to prevent disease recurrence. Historically, transplant-related mortality has been unusually high in this patient population.3,4 Use of reduced-intensity conditioning regimens is associated with improved survival, but this approach has been complicated by a high incidence of mixed chimerism and secondary graft loss in patients with HLH.5,6 Anecdotal evidence has suggested that stable mixed chimerism is sufficient for long-term cure.7,8 However, because clinical data are extremely limited, it remains uncertain whether there is a threshold of donor chimerism above which patients are protected from HLH recurrence.

To better understand how perforin regulates the immune response and how HCT may restore this regulation, we studied the immune reactivity of perforin-deficient (prf−/−) mice engrafted with varying amounts of wild-type (WT) hematopoietic or T cells and challenged with lymphocytic choriomeningitis virus (LCMV) infection. We observed that there was a distinct threshold, 10% to 20% engraftment of WT cells, that reestablished perforin-dependent immunoregulation. Furthermore, our studies also demonstrated that perforin expression in CD8+ T cells was necessary and sufficient to restore normal immune regulation.

Methods

For transplantation, animals were lethally irradiated and injected with 10^7 marrow cells, varying proportions of WT, and prf−/− marrow. WT cells were marked with green fluorescent protein (GFP) or CD45.1. For recombination activating gene (RAG) complementation studies, animals were administered 9 × 10^6 RAG−/− (or RAG/prf−/−) marrow cells along with a mixture of WT and prf−/− marrow (12 to 10%). After waiting 12 to 16 weeks, animals were challenged with LCMV-WE infection (200 plaque-forming units intraperitoneally). For adoptive transfer, magnetically purified (Miltenyi) CD8+ or CD4+ T cells from B6.SJL/BoyJ mice (prf−/− or prf−/−) were transferred 2 days after administering cyclophosphamide (100 mg/kg intraperitoneally). Assessment of serum interferon (IFN)-γ levels, ex vivo dendritic cell antigen presentation assays, and in vivo T-cell IFN-γ measurement was as described.2,9 All studies were repeated at least 3 times with consistent results. All studies were performed on an Institutional Animal Care and Use Committee-approved protocol.
Results and discussion

WT and prf$^{-/-}$ mice display divergent immune responses to LCMV infection. Though WT animals mount a vigorous response, prf$^{-/-}$ animals display unusually strong immune and T-cell activation, which lead to fatal immunopathology. This immune-mediated pathology is widely recognized as an experimental model of HLH. A critical aspect of this pathologic response is prolonged and heightened production of IFN-$\gamma$. To study the effects of marrow transplantation in this model system, we lethally irradiated and reconstituted prf$^{-/-}$ mice with either prf$^{-/-}$ or WT bone marrow. After waiting for immune reconstitution, we challenged these transplanted animals with LCMV infection. As displayed in Figure 1A, we found that transplantation with WT (perforin-expressing) marrow led to an IFN-$\gamma$ response pattern in these animals that was very similar to that seen in intact WT animals. Thus, similar to what is clinically seen, HCT with perforin-expressing marrow restores perforin-mediated immunoregulation in experimental animals. Next, we generated a series of mixed hematopoietic chimeric mice using WT and prf$^{-/-}$ marrow. For these studies, we transplanted a fixed amount of total marrow but varied the proportions coming from each donor. When we plotted the post-LCMV day 8 IFN-$\gamma$ level against the percent of WT chimerism, we observed a distinctive relationship between these 2 parameters (Figure 1B). At low levels of WT chimerism, IFN-$\gamma$ levels were very elevated and similar to those seen in animals with complete prf$^{-/-}$ hematopoiesis. At WT chimerism levels > 10% to 20%, cytokine levels were similar to those observed in animals reconstituted entirely with WT marrow. When we assessed mixed chimeric animals for the development of HLH, we found that those with WT chimerism >20% were protected from the development of severe anemia and death after LCMV infection (supplemental Figure 1, available on the Blood Web site).

We have recently shown that perforin-dependent immunoregulation involves the suppression of antigen presentation and loss of antigen-presenting dendritic cells. To test whether this process was being restored, we sorted and assessed antigen presentation by splenic dendritic cells from LCMV-infected chimeric animals. When we cultured these dendritic cells with transgenic, LCMV-specific T cells, we found that presentation of endogenously acquired viral antigen was suppressed in animals with >10% WT chimerism to levels at or below those seen in intact WT animals (Figure 1C). Thus, a distinct threshold exists (>10% to 20% prf$^{-/-}$ hematopoietic chimerism), above which perforin-dependent immune regulation is reestablished.

Our prior studies and anecdotal clinical data have suggested that T cells play an important role in perforin-mediated immunoregulation. To assess the role of T cells, we generated mice with variable perforin expression in the T-cell and non–T-cell compartments. We reconstituted prf$^{-/-}$ mice with 90% RAG$^{-/-}$/prf$^{-/-}$ or RAG$^{-/-}$/prf$^{-/-}$/WT marrow, along with 10% of a varying mixture of WT and prf$^{-/-}$ marrow. The resulting 3-way chimeric mice developed a T-cell compartment with variable proportions of prf$^{-/-}$ genotype and 90% to 100% of non–T/B cells (myeloid, natural killer, etc) that were either prf$^{-/-}$ or prf$^{-/-}$. When challenged with LCMV, these animals demonstrated that perforin expression in only T cells was sufficient to control cytokine production (Figure 2A). Contribution of WT marrow to non–T (or B) cells ranged from 0% to 3% in most recipients (data not shown). Furthermore, when prf$^{-/-}$/RAG$^{-/-}$ donors were used, giving normal perforin expression in the non–T-cell compartment (>90%), there was no additional effect on IFN-$\gamma$ levels (Figure 2B). Thus, perforin expression in the T-cell compartment is uniquely necessary and sufficient for establishing perforin-dependent immune regulation. To assess the specific role of Foxp3$^{+}$ regulatory T cells and CD1$^{+}$ restricted natural killer T cells, we performed studies using donor marrow or T cells from scurfy, Jalpaha281$^{-/-}$, and CD1$^{+}$ mice and observed similar results as with WT donor marrow, suggesting that neither of these T-cell subsets was critical (supplemental Figure 2).

Finally, we tested the therapeutic effects of adoptive T-cell transfer into intact (nontransplanted) prf$^{-/-}$ mice. To achieve sufficient levels of donor T-cell engraftment, we lympho-depleted recipients with cyclophosphamide before transferring varying numbers of purified WT or prf$^{-/-}$ T cells and then waited 3 weeks for animals to recover before challenging with LCMV. When we transferred WT CD4$^{+}$
T cells, we did not observe a clear trend for rescue (Figure 2C). However, when we transferred purified WT CD8+ T cells (but not prf−/− T cells) (supplemental Figure 2), we observed robust rescue when donor T-cell chimerism exceeded 10% to 20% (Figure 2D). Furthermore, if we examined in vivo IFN-γ production by endogenous (host) CD8+ T cells in recipients, we observed clear suppression in recipients with engraftment of 5% to 10% WT T cells (Figure 2D). Thus, perforin-mediated immune regulation functions in trans; perforin-sufficient cells are able to control the immune hyperactivation observed in perforin-deficient T cells.

We conclude that normal perforin expression in only 10% to 20% of hematopoietic cells, or within the conventional CD8+ T-cell compartment alone, is necessary and sufficient to reestablish normal immune regulation in mice. This finding is an important addition to limited long-term clinical data, consisting of only a few patients with stable donor chimerism in this range. However, additional clinical data are still needed to guide decision making.

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Authorship
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