Perforin is a critical physiologic regulator of T-cell activation

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Individuals with impaired perforin-dependent cytotoxic function (Ctx−) develop a fatal inflammatory disorder called hemophagocytic lymphohistiocytosis (HLH). It has been hypothesized that immune hyperactivation during HLH is caused by heightened infection, defective apoptosis/responsiveness of Ctx− lymphocytes, or enhanced antigen presentation. Whereas clinical and experimental data suggest that increased T-cell activation drives HLH, potential abnormalities of T-cell activation have not been well characterized in Ctx− hosts. To define such abnormalities and to test these hypotheses, we assessed in vivo T-cell activation kinetics and viral loads after lymphocytic choriomeningitis virus (LCMV) infection of Ctx− mice. We found that increased T-cell activation occurred early during infection of Ctx− mice, while they had viral burdens that were identical to those of WT animals, demonstrating that T-cell hyperactivation was independent of viral load. Furthermore, cell transfer and signaling studies indicated that increased antigenic stimulation, not a cell-intrinsic defect of responsiveness, underlay heightened T-cell activation in vivo. Finally, direct measurement of viral antigen presentation demonstrated an increase in Ctx− mice that was proportional to normal T-cell activation. We conclude that perforin-dependent cytotoxicity has an immunoregulatory role that is distinguishable from its pathogen clearance function and limits T-cell activation in the physiologic context by suppressing antigen presentation. (Blood. 2011; 118(3):618-626)

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a unique childhood disorder characterized by predisposing deficiencies of cytotoxic function, excessive immune activation, and potentially fatal damage to the bone marrow, liver, or brain.1,2 Because mutations affecting perforin-dependent cytotoxicity were first found in patients with HLH,3 a variety of speculations have been put forth regarding how they may lead to disease. Most investigators have hypothesized that cell-intrinsic defects of apoptosis,4,5 proliferation,6 or lymphocyte homeostasis7,9 underlie the fatal inflammation seen in humans or mice with defective perforin-dependent cytotoxicity (Ctx−). Implicit in these viewpoints is the assumption that cell death, such as that induced by activation or fratricidal killing, limits T-cell activation (in addition to lymphocyte numbers), although this has never been demonstrated in vivo. In other cases, it has been speculated that persistent or abnormal infections may drive HLH. However, in contrast to individuals with severe combined immune deficiencies, uncontrolled infection is not typically observed to be a cause of mortality in HLH.8,10 An additional long-standing hypothesis relevant to understanding HLH is that cytotoxic lymphocytes may influence antigen presentation by killing APCs. This hypothesis was first proposed by Zinkernagel et al > 20 years ago, when they demonstrated that CD8-dependent depletion of APCs after viral infection led to suppression of heterologous immune responses in WT mice.11-13 Although these studies were not conducted in Ctx− mice, it is hypothesized that defects in such a process could underlie the hyperactive immunity seen in Ctx− individuals. Subsequent studies by several groups have demonstrated that exogenous dendritic cells may be eliminated in vivo in a perforin-dependent fashion.11,12,14-19 However, most of these studies were not conducted in the context of infection and/or they did not directly examine how this process may influence immune activation. Multiple competing hypotheses have been proposed to explain how Ctx− individuals (both humans and mice) develop abnormally intense immune responses, but none of them has been directly tested in Ctx− mice or in the physiologic context of primary viral infection.

Viral infection of Ctx− mice has proven to be a useful context for studying the immune dysregulation underlying HLH. In a manner analogous to Ctx− humans, perforin-deficient (prf−/−) mice develop exaggerated immune responses and/or immune-mediated pathology after a variety of infections.6,20,30 We recognized that the distinctive immunopathology that prf−/− mice develop after lymphocytic choriomeningitis (LCMV) infection is a murine version of HLH, and therefore used this model to demonstrate the critical role of CD8+ T cells and IFN-γ in the development of this disorder.21 Similar HLH-like pathophysiology has subsequently been reported for other Ctx− mice.31,32 Perhaps relevant to this immunopathology, several investigators have reported that prf−/− mice develop increased numbers of virus specific T cells after LCMV infection.6,30 However, increased lymphocyte numbers alone do not explain the pathology of HLH, which instead appears to be largely caused by the toxic effects of excessive inflammation.1,33 Consistent with this clinical observation, our original report and that of Badovinac et al demonstrated that prf−/− mice display abnormally increased T-cell cytokine production after LCMV infection.21,34 However, neither study detailed potential T-cell activation abnormalities or clarified the underlying immune mechanism(s) in Ctx− mice. Therefore, a critical gap remains in understanding how perforin-dependent cytotoxicity shapes immune responses and how deficiencies of this pathway lead to HLH.
In the current study, we tested the competing hypotheses that heightened/prolonged infection, cell-intrinsic defects of T-cell activation, or alterations of antigen presentation underlie the hyperactive immune responses of HLH. We developed several novel methods to study the kinetics of in vivo T-cell activation in Ctx− mice during LCMV infection. Whereas we found that the magnitude of this activation was substantially amplified in Ctx− mice, it was not correlated kinetically with viral burdens in WT and Ctx− mice. Explaining this apparent paradox, transfer studies and direct ex vivo cell-signaling studies revealed that heightened antigenic stimulation was driving exaggerated T-cell activation. Finally, we found that heightened presentation of viral antigens by Ctx− APCs during LCMV infection was proportional to the observed abnormal T-cell activation. We conclude that perforin-dependent cytotoxicity regulates T-cell activation by modulating antigen presentation, in addition to promoting viral clearance.

Methods

Mice and in vivo treatments

Beige, prf−/−, and green fluorescent protein (GFP) transgenic mice were obtained from The Jackson Laboratory and bred in our animal facility. P14 and OTI T-cell transgenic mice were obtained from P. Marrack. Granzyme B cluster-deficient mice were obtained from T. Ley. Jinx mice were obtained from K. Hobbe and B. Beutler. All mice were maintained on the C57BL/6 strain. CD8 T-cell depletion was produced by injecting 1 mg of YTS-169 IP before LCMV infection. For adoptive transfer (Figure 1A), antigen presentation, in addition to promoting viral clearance. For LCMV infection, 200 PFU of LCMV-WE was injected IP or 2 × 10^6 PFU of LCMV clone 13 was injected IV. Unless stated otherwise, “LCMV” refers to LCMV-WE herein. Viral stocks, plasma, and tissues from infected animals were titered using a standard plaque-forming assay.

Virus

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Ex vivo antigen presentation assay

For assessment of splenic APCs, spleens from WT or prf−/− mice were disaggregated in collagenase solution (Liberase; Roche) to free APCs, and endogenous T cells were then depleted from the preparation using magnetic beads (Miltenyi Biotec) before plating at a 3:1 ratio with the indicated T cells.

Flow cytometric assays

Antibodies for flow cytometry were obtained from eBioscience or BioLegend. MHC-peptide tetrameric staining reagents were generated via a recombinant baculoviral system, as described previously. For measurement of APCs, spleens were disaggregated in collagenase solution. For the quantitative comparisons of flow cytometry data, data show representative staining above isotype control staining (or irrelevant tetramer staining) for the indicated population in that specimen.

IVTA assay

For the in vivo T-cell activation (IVTA) assay, ex vivo intracellular cytokine staining was performed (using a method similar to that of Liu et al) by injecting mice with brefeldin-A (100 μg intraperitoneally) and removing spleens 12–18 hours later. Spleens were either disaggregated directly in 1% paraformaldehyde (for IFN-γ staining) or crushed in cold saline solution. For CD107a staining, unfixed cells were incubated at 37°C for 2 hours in medium with 1 μg/mL of brefeldin-A and anti-CD107a (A647-conjugated) antibody. No exogenous antigen was added to the wells. CD8+/CD107− cells were mostly macrophages. For combined tetramer and intracellular cytokine staining, unfixed cells from brefeldin-A–treated mice were incubated at 4°C for 1 hour with tetramer and surface staining before fixation and intracellular staining in 0.03% saponin buffer.

ImageStream analysis

Spleens from LCMV-infected mice were fixed immediately after harvest, permeabilized with ice-cold 100% methanol, and stained for CD8, MHC class II, NFATc1, and DRAQ5. Live-gated, in-focus, single, CD8+ MHCII− cells were selected for ImageStream analysis. The “similarity” feature was used to compare the similarity of the nuclear (DRAQ5) and NFATc1 staining patterns. The similarity feature assigns a numerical value correlating to the degree of overlap of 2 staining patterns, which we refer to as the “nuclear localization index” (NLI) in the case of NFAT staining.

Data analysis/statistics

All studies were repeated at least twice with consistent results and with a minimum of 3 mice per group, although typically more (as indicated). P values were calculated using the Student t test. Error bars displayed in the figures represent SEM.

Results

The magnitude of immune activation is not correlated with viral burden in Ctx− mice

We reported previously that CD8+ T cells in prf−/− mice produce greatly increased amounts of IFN-γ after LCMV infection, which drives HLH-like immunopathology. To determine whether the immune dysregulation we observed in prf−/− mice was unique, we serially measured IFN-γ levels after LCMV infection in additional Ctx− mice. Because cytotoxic function depends on the regulated release of secretory granules containing perforin and granzymes, we examined granzyme B cluster-deficient mice (GrzB−/−) and 2 strains with defective granule exocytosis: beige and Jinx. We found that the normal increase in IFN-γ levels after LCMV infection was greatly heightened in all Ctx− mice at day 6 (Figure 1A). The prf−/− mice exhibited the most severe abnormality, with both an increase at day 6 and a continued increase of cytokine levels through day 8. In general, the severity of cytokine overproduction in Ctx− mice appeared to be correlated with the extent of the cytotoxic defect. This finding mirrors clinical observations in which more severe mutations were associated with worse clinical courses. IFN-γ was largely derived from CD8+ cells, because administration of depleting anti-CD8 antibody to prf−/− mice eliminated > 90% of the detectable cytokine (Figure 1A). These data indicate that cytokine overproduction by T cells is a feature that is common to antiviral immune responses in a variety of Ctx− mutants.

Because Ctx− mice are known to have delayed or absent clearance of LCMV, one could hypothesize that the increased IFN-γ production we observed was simply due to increased viral burden. To test this idea, we measured splenic viral loads in LCMV-infected mice using standard plaque assays at various times. We measured viral loads in beige and prf−/− mice because these represent both mild and severe phenotypes, and confirmed that Ctx− mice do indeed have defective clearance of LCMV at later time points (Figure 1B). However, viral loads were indistinguishable between WT and Ctx− mice before day 10, which encompassed the peak of cytokine production in both WT and Ctx− mice (Figure 1A). Similar viral loads and kinetics were measured...
divergence of cytokine production that was seen with the acutely cleared strain of LCMV (Figures 2A and 1A). Therefore, the responses to 2 very different viral strains revealed a similar immune dysregulation in prf−/− mice. Although expansion and contraction of virus-reactive T-cell populations have been noted to be abnormal in Ctx− mice after persistent infection, the present findings are notable because differential immune activation has not been reported previously in this context. In summary, IFN-γ levels (largely T-cell driven) peak in all mice before viral clearance commences (in WT or Ctx− mice), regardless of whether the virus is acutely controlled or persists at high levels, and this peak is magnified 5-10 fold in Ctx− animals.

In vivo CD8+ T-cell activation is heightened in Ctx− mice after LCMV infection

Whereas increased levels of T-cell–derived cytokines suggest an increase in T-cell activation, they are only an indirect measure. To directly assess T-cell activation kinetics in vivo, we developed a technique similar to that described by Liu et al., which we term the IVTA assay. In this method, brefeldin-A is administered to virally infected animals and 12-18 hours later spleen cells are fixed immediately ex vivo and stained intracellularly for IFN-γ without in vitro stimulation. Therefore, the IVTA assay measures the number of T cells that are actually producing cytokine in vivo, not the number that are capable of producing cytokine if stimulated in vitro, which is what conventional intracellular cytokine/ELISpot

Figure 2. Prf−/− mice display heightened immune activation independently of virus control. (A) Serum IFN-γ levels were measured in WT and prf−/− mice infected with the persistent strain of LCMV, clone 13 (LCMV-C13), at the indicated time points. Serum IFN-γ levels at days 6, 8, and 10 were significantly elevated (P < .01) in prf−/− mice compared with WT. (B) Plasma viral levels were measured by plaque assay at the indicated time points in LCMV-C13–infected mice. Dashed line indicates limit of detection of the assay. *P < .01.
Therefore, the increased number of cytokine-producing T cells has a similar ability to produce the cytokine (Figure 3B). Using the IVTA assay, we found that prf-/- mice harbored up to 5-fold more IFN-γ+, CD8+ T cells (compared with WT) during the peak of the antiviral response, which is roughly proportional to serum IFN-γ levels (Figure 3A). CD8+ T cells were more likely to produce IFN-γ in prf-/- mice in both percentage and absolute terms. IFN-γ+ CD8+ T cells in Ctx- mice also appeared to be highly activated, because they had a blasting morphology (increased forward scatter) and were largely CD25+ (data not shown). Whereas other investigators have demonstrated an increase in the total number of antiviral T cells after infection of Ctx- mice, this substantial increase in the number of activated T cells in Ctx- mice after a primary viral infection is novel. One possible explanation for this increased activation is that Ctx- CD8+ T cells are intrinsically more “reactive.” However, when we transgenic T cells (P14 or OTI) that were either prf1/1 or prf1/- in vitro with cognate peptide-loaded dendritic cells, we found no differences in IFN-γ production, suggesting that Ctx- T cells have a normal responsiveness to antigenic stimulation (data not shown).

Although the IFN-γ-secreting T cells we observed in prf-/- mice were presumably responding to LCMV, we established the specificity of these cells by combining the IVTA assay with peptide-MHC tetramer staining for a dominant LCMV-derived epitope, Gp33-41 (Db restricted, thus Db-Gp33). We did this by briefly staining fresh cells from brefeldin-treated mice with peptide-MHC tetramer at 4°C before fixation and permeabilization. We found that approximately 32% of Gp33-specific T cells in prf-/- mice were producing IFN-γ 8 days after LCMV infection, whereas only 8% of the corresponding cells in WT mice were (Figure 3B). As a positive control, we injected Gp33-41 peptide IV into additional LCMV-infected animals 4 hours before killing. Peptide injection greatly increased the percentage of Gp33-specific T cells that were producing IFN-γ in both types of mice, and demonstrated that they had a similar ability to produce the cytokine (Figure 3B). Therefore, the increased number of cytokine-producing T cells found in prf-/- mice is due to the increased probability that antiviral T cells have experienced recent activation, presumably because of antigen encounter. Furthermore, IFN-γ+ cells do not appear to be bystanders, because when we transferred ovalbumin-specific T cells (OTI) into LCMV-infected WT and prf-/- mice, these irrelevant cells did not become IFN-γ+ (data not shown).

In addition to cytokine staining, we sought an additional corroborating measure of in vivo T-cell activation by measuring ex vivo degranulation of CD8+ T cells. Similar to the IVTA assay, at various times after infection we administered brefeldin-A directly to animals, removed spleen cells, and incubated them briefly ex vivo (without the addition of antigen) in the presence of anti-CD107a antibody to label cells that were spontaneously degranulating and exposing this antigen. Exposure of CD107a after stimulation with target cells has been widely used as a measure of cytotoxic degranulation by T cells and natural killer (NK) cells. This application of the assay is unique, however, because we were measuring degranulation without the addition of exogenous antigen. Using this approach, we were able to quantitate the number of T cells that were degranulating (CD107a+) in vivo at various times after LCMV infection. Similar to the cytokine staining data above, we found that prf-/- mice had significantly increased numbers (approximately 3-fold) of CD107a+ CD8+ cells during the peak of T-cell activation (Figure 3C). This finding further suggests that
antigenic stimulation drives heightened T-cell activation in Ctx− mice, because degranulation by T cells, like cytokine production, is thought to be tightly linked to stimulation via the TCR.

Viral-specific CD4+ T-cell expansion and in vivo activation is heightened in Ctx− mice after LCMV infection

If increased CD8+ T-cell activation is due to increased antigen presentation, then one would predict that CD4+ T-cell populations would also be affected by such an abnormality. To test this prediction, we assessed CD4+ T-cell responses after LCMV infection in WT and prf−/− mice in 2 ways. First, we used a peptide-MHC tetramer staining reagent (IAb-Gp61) to measure the endogenous anti-LCMV CD4+ T-cell response to a dominant LCMV epitope, and found that this population was almost 5-fold larger in prf−/− mice by day 8 of LCMV infection (Figure 4A). Second, we measured the number of CD4+ T cells producing IFN-γ in vivo using the IVTA assay, and found a nearly 5-fold increase in these cells by day 8 after LCMV infection (Figure 4B). Therefore, both the development of CD4+ T-cell antiviral responses and activation of these cells is enhanced in prf−/− mice. We also examined beige mice and found a similar enhancement in both parameters (data not shown). The changes in the CD4+ compartment were similar to those observed with CD8+ T cells. These findings are also notable because most CD4+ T cells do not express perforin, suggesting that perforin-expressing cells may regulate the activation of other cell populations (such as CD4+ T cells), in trans.

CD8+ T-cell populations from prf−/− mice display cell-signaling patterns indicating heightened antigenic stimulation after viral infection

Because heightened T-cell cytokine production and degranulation in Ctx− mice suggest increased antigen stimulation,42-44 we predicted that T cells from these animals should display evidence of recent signaling through the TCR. Consistent with this prediction, we found that IFN-γ+ T cells from prf−/− mice displayed increased levels of phospho-c-Jun (Figure 5A). Although accumulation of phospho-c-Jun in T cells suggests recent TCR-driven ERK signaling, we sought additional specific indicators of recent TCR signaling. Nuclear factor of activated T cells (NFAT) is well-described as an integrator of signals from the TCR and relevant costimulatory receptors, including highly transient signaling phenomena lasting only seconds, such as calcium concentration spikes.45,46 When such activating signals are received by T cells, NFAT isoforms translocate from the cytoplasm to the nucleus, where they induce gene expression. Although NFAT translocation is a complex, highly regulated process, the accumulation of NFAT in the nucleus strongly implies recent antigenic stimulation of a T cell through the TCR. Therefore, we quantitated relative nuclear localization of NFATc1 in CD8+ T cells directly ex vivo from LCMV-infected WT and prf−/− mice using a flow cytometric imaging device (ImageStream). NLI was calculated by comparing the similarity of nuclear and NFAT staining patterns within CD8+ cells. As Figure 5 illustrates, the proportion of CD8+ T cells with nuclear-localized NFATc1 was increased on day 8 after LCMV infection in all mice. However, cells with substantial nuclear localization of NFAT (NLI of > 2) were significantly increased in infected prf−/− mice compared with infected WT mice (Figure 5B-D). This difference was similar to what we observed with other measures of recent T-cell activation, although nuclear-localized NFAT was more frequent than IFN-γ staining in WT T cells. These cell-signaling patterns, along with increased IFN-γ production and increased degranulation of CD8+ T cells, strongly point to increased antigenic stimulation as the proximal factor driving abnormal T-cell activation in Ctx− mice.

Increased T-cell activation in Ctx− mice after infection is due to heightened antigen presentation

Because our findings suggested that T cells in Ctx− mice were responding to external antigenic cues and were not intrinsically hyperresponsive, we predicted that WT and Ctx− T cells would exhibit similar activation in the same environment. To test this prediction, we transferred a small number of naive, polyclonal CD8+ T cells from WT or prf−/− mice (transgenically marked with GFP) into WT or prf−/− hosts and then infected them with LCMV. Six days later, we assessed in vivo IFN-γ production by donor (and host-derived) cells via the IVTA assay. We found that T cells transferred into prf−/− mice were nearly 5-fold more likely to
produce IFN-γ in vivo regardless of whether they came from prf−/− mice (Figure 6A). In addition, the percentage of IFN-γ cells in the transferred population approximated the percentage of endogenous cells that were IFN-γ in both WT and prf−/− recipients (Figure 3 and data not shown). Therefore, even though prf−/− CD8+ T cells have an intrinsic defect of cytotoxic function, their abnormal activation after LCMV infection is due to cell-extrinsic factors in Ctx− mice. The transferred cells comprised <1% of total CD8+ T cells in the host, and no effect was noted on serum IFN-γ levels or viral loads (data not shown).

Because our data suggested that T cells in Ctx− animals were responding normally to heightened antigen presentation, we predicted that we would observe significant differences in APC populations between LCMV-infected WT and prf−/− mice. When we measured splenic APC populations 7 days after LCMV infection, we found that there were substantial changes in all mice, including decreases in CD8+ dendritic cells (CD8+/CD11c− cells, which are thought to be especially important for priming CD8+ T cells). The transferred cells comprised 1% of total CD8+ T cells in the host, and no effect was noted on serum IFN-γ levels or viral loads (data not shown).
Table 1. Increases in T-cell numbers, activation indices, and antigen presentation in prf−/− compared with WT mice after LCMV infection

<table>
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<tr>
<th>Increases, fold</th>
<th>Antigen-specific T cells, no.</th>
<th>T-cell-activation indices</th>
<th>Viral antigen presentation by splenic APCs</th>
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<tbody>
<tr>
<td>CD8+ T cell</td>
<td>2.5 (Db-Gp33+)</td>
<td>6.1 (IFN-γ+) 2.6 (CD107+)</td>
<td>4.6 Gp33, presented by Db</td>
</tr>
<tr>
<td>CD4+ T cell</td>
<td>5.2 (IAb-Gp61+)</td>
<td>4.8 (IFN-γ+)</td>
<td>Not assessed</td>
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The fold change was calculated by dividing the indicated value observed in prf−/− mice by that observed in WT animals after LCMV infection. All indices were assessed 8 days after infection, except antigen presentation, which was assessed 7 days after infection.

Db-Gp33+ and IAb-Gp61+ refer to the absolute number of CD8 and CD4 T cells staining with these tetramers per spleen. IFN-γ+ and CD107+ refer to the absolute number of such cells per spleen detected using direct ex vivo staining.

Discussion

Although defective perforin-dependent cytotoxic function has been known to underlie “hyperinflammation” in the human disorder HLH for over a decade, the immunoregulatory nature of cytotoxic function remains poorly defined in physiologic contexts. In the current study, we tested 3 principal hypotheses about how deficiencies of perforin-dependent cytotoxic function may lead to excessive immune activation. First, we found that immune hyperactivation is not correlated with increased or persistent pathogen burden in Ctx− mice. Whereas this does not rule out aberrant infection as a potential contributor to disease pathology in patients with HLH, it does demonstrate that perforin-dependent cytotoxic function has a physiologic immune regulatory function that can be distinguished from its role in pathogen clearance. Second, we found that increased T-cell activation in Ctx− mice is not due to cell-intrinsic defects of activation. Although T cells and NK cells in Ctx− mice do have cell-intrinsic defects of cytotoxic function, this does not appear to directly cause abnormal lymphocyte activation. Third, we found that increased presentation of viral antigen appears to be the principal cause of heightened T-cell activation in Ctx− mice after LCMV infection. However, whether specific APCs are directly killed or otherwise altered in a perforin-dependent fashion remains to be defined.

The findings of the current study, along with our previous study in which we demonstrated that HLH disease pathology in prf−/− mice is driven by activated CD8+ T cells, demonstrate that perforin-dependent cytotoxicity limits life-threatening immunopathology by regulating T-cell activation. Our findings suggest that cytotoxic lymphocytes (CD8+ T cells or NK cells) shape the developing immune response by suppressing antigen presentation. However, the specific identity of the cytotoxic cells altering antigen presentation remains unclear. It is possible that multiple cell types may exert perforin-dependent immune regulation. Recent studies have suggested that NK cells may affect T-cell responses in physiologic contexts by affecting antigen presentation. In addition, regulatory T cells have been demonstrated to alter dendritic cell populations in a perforin-dependent fashion.

Our results also suggest that perforin-dependent cytotoxicity alters APC populations in subtle ways. Although other investigators have noted perforin-dependent killing of exogenously administered dendritic cells in vaccine contexts, we did not find a global loss of endogenous dendritic cells or macrophages in WT mice compared with Ctx− mice. Despite this, there was a clear shift in viral antigen presentation. Whereas it is possible that nonprofessional APCs are driving T-cell activation in Ctx− mice, it seems more likely to us that functional shifts or losses of small subpopulations of APCs are responsible for the changes we observed. Because very little is known about physiologic antigen presentation to T cells at late time points after infection, further study will be required to determine how perforin alters APC populations and their function in the physiologic context.

The phenotype of human cytotoxic deficiency is unique among primary immune deficiencies in that it does not lead to chronic or unusual infections, but instead leads to the dangerous hyperinflammation known as HLH. The fundamental importance of this form of immune regulation for humans is illustrated by the fact that infants with deficiencies of cytotoxic function may develop fatal immunopathology as severe as (albeit different from) infants with immunodysregulation polyendocrinopathy enteropathy X-linked syndrome (IPEX), a natural deficiency of classic CD4/CD25+ regulatory T cells. Unlike other experimental studies of perforin-dependent cytotoxic function, which have largely focused on perforin’s importance for host resistance, the present study focused on the immunoregulatory role of perforin that is uniquely valuable for understanding human immunodeficiency. The immunemediated pathology that develops in Ctx− hosts appears to be unique in that it is T-cell driven, yet is not autoimmune or allergic.
These findings also have significant translational implications for the treatment of patients with HLH. The current standard of care for the treatment of HLH consists of an empirically derived etoposide/dexamethasone–based regimen (with the addition of cyclosporine being tested in an ongoing clinical trial). In addition, successful outcomes with an antithymocyte globulin/prednisone–based regimen have been reported. Our current results suggest that HLH should be conceptualized as a disease of excessive T-cell activation that occurs in response to enhanced (and perhaps also prolonged) antigen presentation. Therefore, this study provides clear impetus for the development of novel therapeutic approaches directed at activated T cells, APCs, and the interface between them. A readily achievable clinical translation of these findings would include further testing of serotherapies directed at eliminating T cells, such as antithymocyte globulin or alemtuzumab, which have not yet been tested in large multicenter trials. Our findings support further investigation of these drugs, perhaps in rationally designed combinations with etoposide. In addition, multiple immune-modulating drugs affecting T-cell activation are currently approved or are in clinical trials, but have never been tested in patients with HLH. The results of the current study provide clear rationale for preclinical and clinical investigation of these agents. Finally, no approved or investigational drugs are known to specifically suppress antigen presentation. Our data provide a rationale for testing novel candidate drugs that may directly suppress antigen presentation. Such agents may mimic perforin–dependent immune regulation that is missing in patients with HLH. Deeper understanding of the immune dysregulation underlying HLH is likely to lead to improved therapies for patients with this disorder.

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Authorship

Contribution: J.E.L. and M.B.J. designed experiments; J.E.L., C.E.T., E.E.Z., and M.B.J. conducted experiments; and M.B.J. and K.R. wrote the manuscript.

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