Disruption of MyD88 signaling suppresses hemophagocytic lymphohistiocytosis in mice

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Hemophagocytic lymphohistiocytosis (HLH) is a rare inflammatory disorder with a poor prognosis for affected individuals. To find a means of suppressing the clinical phenotype, we investigated the cellular and molecular mechanisms leading to HLH in *Unc13d[jinx/jinx]* mice, in which cytolytic function of NK and CD8^{+} T cells is impaired. *Unc13d[jinx/jinx]* mutants infected with lymphochoriomeningitis virus (LCMV) present typical clinical features of HLH, including splenomegaly, elevated serum IFNγ, and anemia. Proteins mediating cell-cell contact, cytokine signaling or Toll-like receptor (TLR) signaling were analyzed. We show that neither the integrin CD18, which is involved in adhesion between antigen-presenting cells and effector T cells, nor tumor necrosis factor (TNF) made nonredundant contributions to the disease phenotype. Disruption of IFNγ signaling reduced immune cell activation in *Unc13d[jinx/jinx]* mice, but also resulted in uncontrolled viral proliferation and exaggerated release of inflammatory cytokines. Abrogating the function of myeloid differentiation primary response gene 88 (MyD88) in *Unc13d[jinx/jinx]* mice suppressed immune cell activation and controlled cytokine production in an IL-1 receptor 1 (IL-1R1)–independent way. Our findings implicate MyD88 as the key initiator of myeloid and lymphoid proliferation in HLH, and suggest that blockade of this signaling molecule may reduce immunopathology in patients. (*Blood*. 2011;117(24): 6582-6588)

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a rare disorder of the immune system. The familial form (FHL) arises in children and is caused by mutations that impair the cytotoxic activity of NK cells and CD8^{+} T cells. The second form, sporadic HLH, may arise in individuals with pathogen infections or with autoimmune diseases or malignancies.[1] Both HLH variants are believed to be initiated by viral infections.[2] These pathogenic triggers have remained elusive, although Epstein-Barr virus has been proposed as one cause.[3-4]

After symptomatic onset of HLH, survival declines rapidly despite chemotherapy with cytotoxic and immunosuppressive drugs.[5] To date, hematopoietic stem cell transplantation is the best long-term therapy for familial HLH.

We previously described an N-ethyl-N-nitrosourea (ENU)–germ line mutant called *jinx* in which a mutation in *Unc13d* prevents the release of cytolytic granules by NK cells and CD8^{+} T cells.[6] We demonstrated that clinical features of the HLH-like syndrome observed in humans are recapitulated in *Unc13d[jinx/jinx]* mice infected with lymphochoriomeningitis virus (LCMV). *Unc13d[jinx/jinx]* remains the only animal model of human type 3 FHL, which is caused by mutations in MUNC13-4, the human ortholog of *Unc13d*.

On infection of *Unc13d[jinx/jinx]* mutants, a positive feedback loop is initiated between CD8^{+} T cells and antigen-presenting cells (APCs) such as macrophages, leading to their overactivation and HLH-like symptoms.[6] We hypothesized that interrupting this positive feedback loop could ameliorate the onset and/or extent of HLH-like disease and potentially reveal novel therapeutic targets

for human HLH. We therefore examined the impact of disrupting cell adhesion molecules, effector cytokines, and innate immune sensors on the progression of HLH-like disease in *Unc13d[jinx/jinx]* mutants, and identified myeloid differentiation primary response gene 88 (MyD88) as a key protein required for the development of the syndrome.

Methods

Mice

All mouse studies were performed in accordance with institutional regulations governing animal care and use. C57BL/6J mice were bred locally at The Scripps Research Institute. The following mutants have been previously reported and are described at http://mutagenetix.scripps.edu: *Unc13d[jinx/jinx]* [6] (MGI: 3628822; MMRRC: 016137-UCD), *Myd88[poc/poc]* [7] (MGI: 3641255; MMRRC:010475-UCD), and *Tnf[PanR1/PanR1]* [8] (MGI: 3616888; MMRRC:010462-UCD). *Itgb2[Joker/Joker]* mice (MGI:3808883; MMRRC:016138-UCD) carry an A to T transversion in the acceptor splice site of intron 6 (TCCCAG→TCCCTG) in the *Itgb2* gene, resulting in a complete deletion of exon 7. The mutated CD18 protein cannot associate with CD11a, CD11b, or CD11c.[9] Interferon γ receptor (IFNγR)–deficient mice were kindly provided by Dr Charles D. Surh (The Scripps Research Institute) and B6.129S7-*Il1r1[tm1Imx]*/J, mice were obtained from The Jackson Laboratory. Compound mutants were generated by intercrossing and genotyping the respective F1 progeny.

Figure 1. Lack of anemia and leukocyte hyperproliferation in wild-type and *Unc13d^jinx/jinx;Myd88^poc/poc* mice after infection. Blood samples and spleens were collected from control and double mutant mice at day 12 after LCMV infection. Circulating red blood cells (A) and white blood cells (B) and total splenocytes (C) were enumerated. *jinx, Unc13d^jinx/jinx* mice; *jinx; Myd88^poc, Unc13d^jinx/jinx;Myd88^poc/poc* mice. Representative data of 3 independent experiments are shown; n = 5 per group. Error bars show SEM.
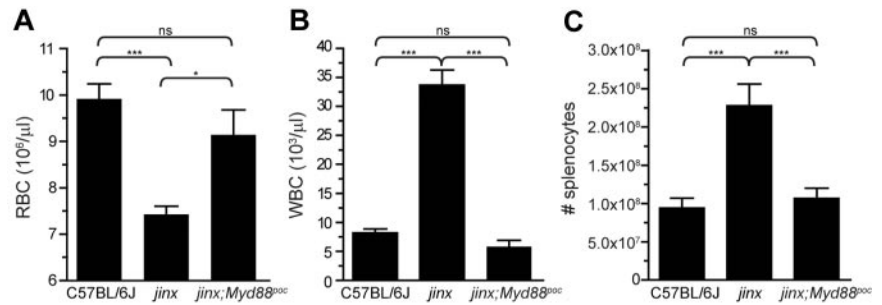
Complete blood counts and serum cytokine detection

Blood samples were taken from the retro-orbital plexus of mice and analyzed using a Hemavet 950 veterinary hematology system (Fisher Scientific). Alternatively, IFNγ or TNF were assayed in the serum by ELISA (eBioscience).

Viruses, focus-forming unit assay

The Armstrong strain of LCMV was injected intravenously at a dose of 2×10^5 plaque-forming units per mouse. Viral titers were determined after organ homogenization by standard plaque assays on VERO cells.

Antibodies and intracellular cytokine staining

The following antibodies were used for flow cytometry, to stain splenocytes: CD11b (M1/70), CD3ε (145-2C11), CD8α (53-6.7), CD80 (B7-1), CD86 (GL1), F4/80 (BM8), IFNγ (XMG1.2), TNF (MP6-XT22). Specific CD8+ T-cell responses were determined 12 days after infection, from splenocytes, by intracellular IFNγ and TNF staining after a 5-hour stimulation with 10^{-7} M LCMV GP33 (KAVYNFATM) peptide as described before.[6] Specific cytokine secretion after stimulation with LCMV-derived peptide was measured as (% cytokine+ CD8+ T cells after peptide stimulation) − (% cytokine+ CD8+ T cells after stimulation without peptide). For spontaneous cytokine production, % cytokine+ CD8+ T cells after stimulation without peptide are indicated.

Histology

For histologic analysis, tissues were fixed in 10% buffered formalin. Paraffin-embedded sections were stained with H&E.

Statistics

The statistical significance of differences was determined by unpaired Student 2-tailed t test. Differences with a P < .05 were considered statistically significant. For all figures: ns indicates not significant; *P < .05; **P ≤ .01; ***P ≤ .001.

Results

Disruption of CD18 does not affect HLH-like syndrome

Lymphocyte-function associated antigen (LFA-1) is a heterodimeric integrin that is involved in lymphocyte migration and intercellular adhesion. Because LFA-1 is found at the immunologic synapse between LCMV-infected target and CD8+ T cells,[10] we hypothesized that impairing its function would restrain uncontrolled CD8+ T-cell proliferation in LCMV-infected *Unc13d^jinx/jinx* mice. To test this, we bred *Unc13d^jinx/jinx* mutants with *Joker* mice, in which a point mutation in the *Itgb2* gene precludes surface expression of CD18, the β chain of the LFA-1 complex.[9]

Despite a decreased response on day 7 after LCMV infection, when T-cell expansion peaks (supplemental Figure 1A, available on *Blood* Web site; see the Supplemental Materials link at the top of the online article), the LCMV GP33-specific T-cell response in *Unc13d^jinx/jinx;Itgb2^Joker/Joker* mice was as severe as in *Unc13d^jinx/jinx* mice on day 12, when T cells usually contract (supplemental Figure 1B-C). The *Joker* mutation did not alter the spontaneous production of IFNγ in *jinx* CD8+ T cells 7 days after infection (supplemental Figure 1D). A similar differential behavior among CD8+ T-cell populations has been previously reported in LCMV-infected, CD18-deficient mice around the peak of the T-cell response.[11] On day 12 after infection, *Unc13d^jinx/jinx;Itgb2^Joker/Joker* CD8+ T cells accumulated, produced IFNγ spontaneously and displayed an activated phenotype (supplemental Figure 1C-E). Furthermore, both *Unc13d^jinx/jinx;Itgb2^Joker/Joker* and *Unc13d^jinx/jinx* mice displayed hematologic symptoms of HLH-like disease on day 12, namely low hematocrit, leukocytosis and neutrophilia (supplemental Figure 1F-H, respectively). Therefore, inhibiting integrin-dependent interaction is not sufficient to dampen the later progression of HLH-like syndrome in *Unc13d^jinx/jinx* mice.

The development of HLH-like syndrome is TNF-independent but IFNγ-dependent

HLH-affected individuals exhibit elevated concentrations of circulating cytokines including TNF and IFNγ, which are indicative of a poor clinical outcome in young patients.[12-13] We evaluated the role of these cytokines in the development of experimental HLH. To this end, *Unc13d^jinx/jinx* mutants were bred to *Tnf^PanR1/PanR1* mice, in which TNF bioactivity is completely abrogated,[8] and to IFNγ receptor (IFNγR)–deficient mice. On day 12 after LCMV infection, *Unc13d^jinx/jinx* and *Unc13d^jinx/jinx;Tnf^PanR1/PanR1* mice had enlarged spleens (supplemental Figure 2A), with elevated proportions of CD8+ T cells and macrophages (supplemental Figure 2B-C). By contrast, *Unc13d^jinx/jinx;Ifngr^−/−* mice spleens did not develop splenomegaly and the proportions of splenic CD8+ T cells and macrophages were comparable with those observed in wild-type mice (supplemental Figure 2B-C). However, circulating levels of IFNγ and TNF in *Unc13d^jinx/jinx;Ifngr^−/−* mice were approximately 40-fold and 5-fold higher than in *Unc13d^jinx/jinx* controls on day 12 after LCMV infection (supplemental Figure 2D and E, respectively). A substantial elevation in the concentration of serum IFNγ was already observable in *Unc13d^jinx/jinx;Ifngr^−/−* mice relative to *Unc13d^jinx/jinx* mice on day 7 after LCMV infection (supplemental Figure 2F).

These data demonstrate that TNF signaling has no major role in the development of HLH-like syndrome in LCMV-infected *Unc13d^jinx/jinx* mice, whereas IFNγ/IFNγR signaling is required for uncontrolled APC and CD8+ T-cell proliferation. *Unc13d^jinx/jinx;*
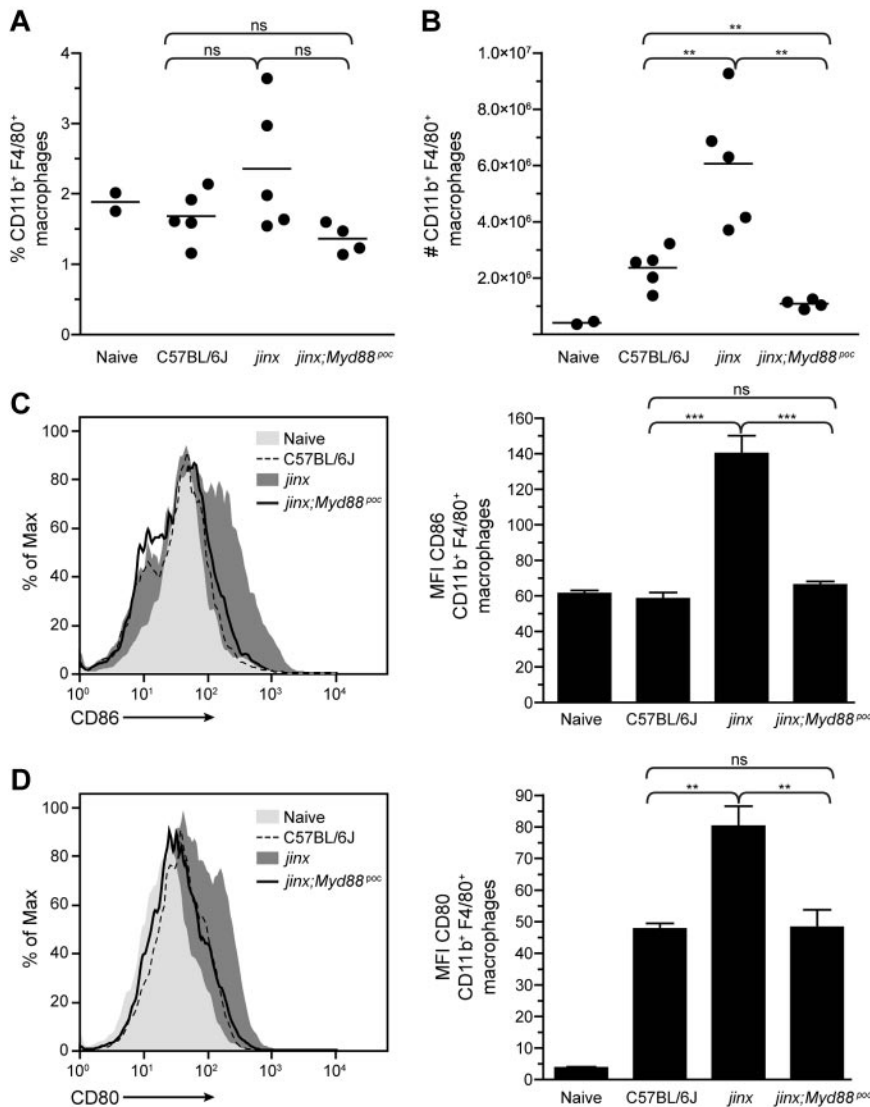
Figure 2. Absence of proliferation and expression of costimulatory molecules by $Unc13d^{jinx/jinx}$; $Myd88^{poc/poc}$ macrophages after infection. Twelve days after LCMV infection, splenocytes from mice were stained for CD11b and F4/80 expression to evaluate the percentage of activated macrophages (A) and their absolute numbers (B). Each dot represents an individual mouse. Histograms in panels C and D show the surface expression of CD86 and CD80 on CD11b$^+$F4/80$^+$ macrophages, respectively. The respective MFI are reported on associated graphs. jinx, $Unc13d^{jinx/jinx}$ mice; jinx; $Myd88^{poc}$, $Unc13d^{jinx/jinx};Myd88^{poc/poc}$ mice. Representative data of 3 independent experiments are shown for panels A and B and 2 independent experiments for panels C and D; $n \geq 4$ per group of infected mice. Error bars show SEM.

$Ifngr^{-/-}$ mice became hunched and weak by day 12 after infection, with 100 000-fold higher viral loads in their kidneys than $Unc13d^{jinx/jinx}$ mice (supplemental Figure 2G); LCMV infection led to a fatal outcome in some of these mice (data not shown). Unresolved viral proliferation in the compound mutants is consistent with previous studies showing that IFNγ-deficient mice are susceptible to LCMV[14] and that elevated viral loads in $Ifngr^{-/-}$ mice are further aggravated by the absence of perforin.[15-16] In addition, administration of an IFNγ-blocking antibody to perforin-deficient mice during the peak CD8$^+$ T-cell response to LCMV resulted in remission of HLH-like symptoms.[17-18] Despite the use of different LCMV strains, these complementary observations suggest that the production of IFNγ at early times during viral infection may be critical to generate an initial antiviral state by priming immune responses in HLH prone individuals, before high quantities of IFNγ secreted by activated CD8$^+$ T cells trigger the development of immunopathology. In our model of IFNγR-deficiency, this early antiviral state may be prevented, resulting in increased viral susceptibility. Early blockade of IFNγ/IFNγR signaling may therefore be deleterious in HLH-affected individuals. Alternatively, and as previously reported in another model of virus infection, deficiency of IFNγR may result in a different effect than deficiency of its ligand, IFNγ.[19]

Disrupting MyD88 signaling abolishes the development of HLH-like syndrome

TLRs are sensors of pathogens, and most TLRs recruit the adaptor MyD88 on stimulation. During LCMV infection, MyD88 expressed by APCs is implicated in proinflammatory cytokine production[20] and CD8$^+$ T-cell expansion,[21-22] 2 hallmarks of HLH. To examine the effects of a disruption of MyD88 signaling in the development of HLH-like disease in $Unc13d^{jinx/jinx}$ mice, we introduced the *pococurante* (*poc*) allele of $Myd88$[7] onto the $Unc13d^{jinx/jinx}$ background. LCMV-infected $Unc13d^{jinx/jinx};Myd88^{poc/poc}$ mice and wild-type controls displayed comparable densities of red blood cells (RBCs) and white blood cells (WBCs) on day 12 (Figure 1A-B). LCMV infection did not affect hematocrit, hemoglobin, circulating lymphocytes, neutrophils, and monocytes in $Unc13d^{jinx/jinx};Myd88^{poc/poc}$ mice (supplemental Figure 3), and the spleen cellularity of these double mutants was half that observed in $Unc13d^{jinx/jinx}$ mice (Figure 1C). Moreover, whereas the proportion of splenic macrophages in $Unc13d^{jinx/jinx}; Myd88^{poc/poc}$ mice was comparable with the one in $Unc13d^{jinx/jinx}$ mice after infection (Figure 2A), the absolute number of these cells was 6-fold lower than in $Unc13d^{jinx/jinx}$ mice (Figure 2B). $Unc13d^{jinx/jinx};Myd88^{poc/poc}$ macrophages did not

Figure 3. Absence of hyperproliferation and overactivation of *Unc13d$^{jinx/jinx}$;Myd88$^{poc/poc}$* CD8$^+$ T cells on day 12 after LCMV infection. Splenocytes from mice were stained to evaluate the percentage of CD8$^+$ T cells (A) and their absolute numbers (B). Each dot represents an individual mouse. (C) Graph reporting the percentages of CD8$^+$ T cells that spontaneously secrete IFNγ after 5-hour incubation in media without peptide stimulation. (D-E) IFNγ production by CD8$^+$ T cells after stimulation with LCMV GP33. The percentage of IFNγ$^+$ cells among the CD8$^+$ T-cell population is indicated in representative dot plots (D). Absolute counts of LCMV GP33-specific IFNγ$^+$CD8$^+$ T cells are shown in panel E. (F) Histogram reporting the percentages of LCMV GP33-specific CD8$^+$ T cells that secrete either IFNγ alone (white rectangles) or IFNγ and TNF (black rectangles). Representative data of 3 independent experiments are shown for (A-F); n ≥ 4 per group of infected mice. Error bars show SEM.
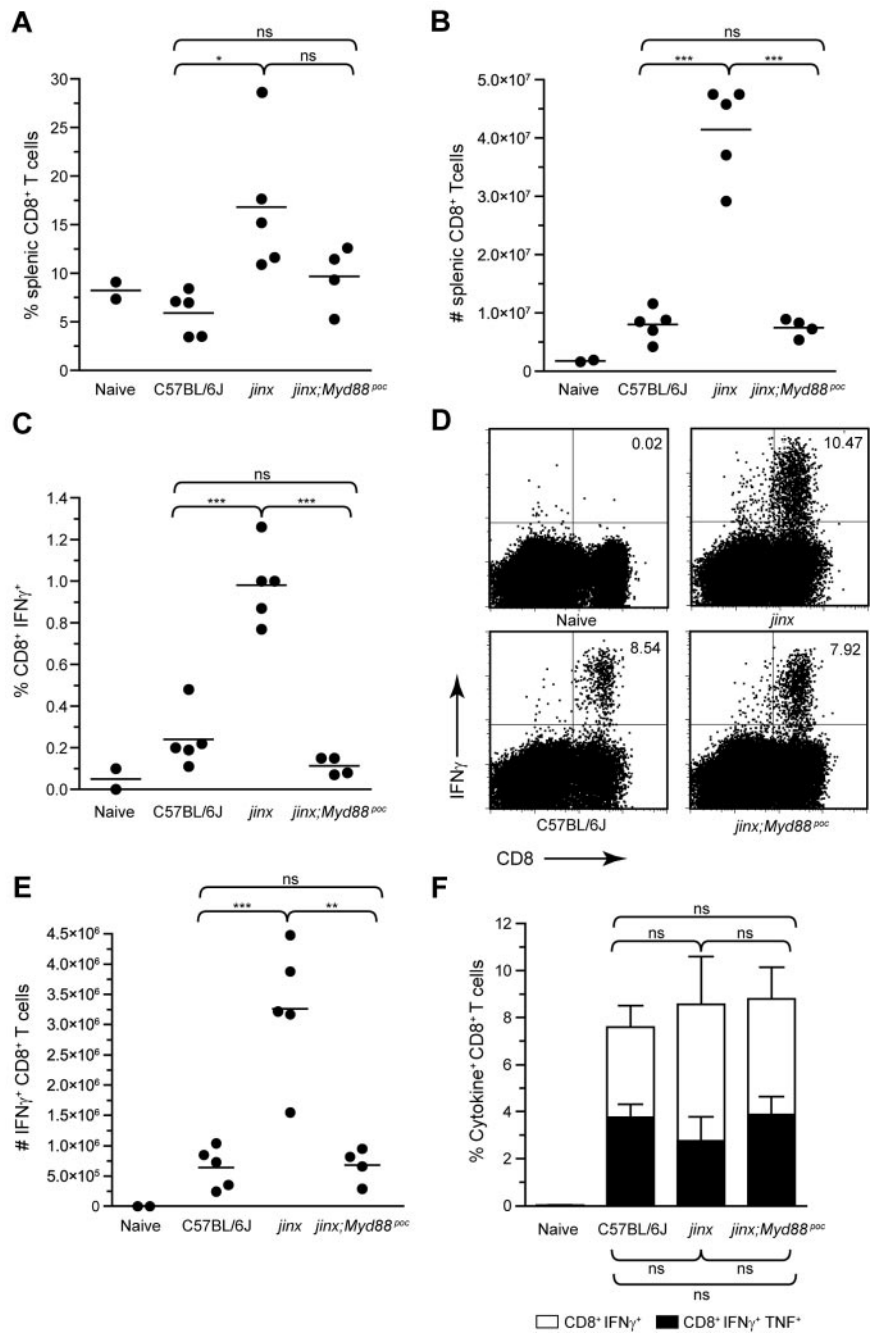
up-regulate CD86 (Figure 2C) and CD80 (Figure 2D) on their surface as opposed to *Unc13d$^{jinx/jinx}$* macrophages. Because MyD88 is not involved in the up-regulation of CD86 and CD80 on APCs[20] or in antigen presentation in LCMV-infected wild-type mice,[22] we hypothesize that the down-modulation of these molecules at the surface of *Unc13d$^{jinx/jinx}$;Myd88$^{poc/poc}$* macrophages is cell-extrinsic and possibly attributed to an indirect effect mediated by other cells.

As the ablation of MyD88 signaling in the *Unc13d$^{jinx/jinx}$* background affects the expression of costimulatory molecules on APCs, T-cell activation in *Unc13d$^{jinx/jinx}$;Myd88$^{poc/poc}$* mice may be dampened as well. The proportions and the absolute numbers of splenic CD8$^+$ T cells in *Unc13d$^{jinx/jinx}$;Myd88$^{poc/poc}$* mice and wild-type mice were similar (Figure 3A-B). The percentage of CD8$^+$ T cells that spontaneously produced IFNγ in wild-type and *Unc13d$^{jinx/jinx}$;Myd88$^{poc/poc}$* mice were comparable and approxi-

mately one-tenth the percentage observed in *Unc13d$^{jinx/jinx}$* mice on day 12 after infection (Figure 3C). In vitro stimulation of splenocytes with the LCMV-derived peptide GP33 showed that the total percentage of IFNγ-producing CD8$^+$ T cells (Figure 3D) and their absolute numbers (Figure 3E) were high in *Unc13d$^{jinx/jinx}$* mice, but significantly lower and equivalent to wild-type in *Unc13d$^{jinx/jinx}$; Myd88$^{poc/poc}$* mice. The percentage of LCMV GP33-specific cytokine-producing CD8$^+$ T cells was comparable in all strains (Figure 3F).

Taken together, CD8$^+$ T-cell responses in *Unc13d$^{jinx/jinx}$; Myd88$^{poc/poc}$* mice were lower than in *Unc13d$^{jinx/jinx}$* mutants, consistent with the reduced activation state of their macrophages. Therefore, inhibition of MyD88 signaling in the *Unc13d$^{jinx/jinx}$* background not only prevented overproliferation of immune cells, but also dampened antigen-presenting cell maturation and overactivation of CD8$^+$ T cells.
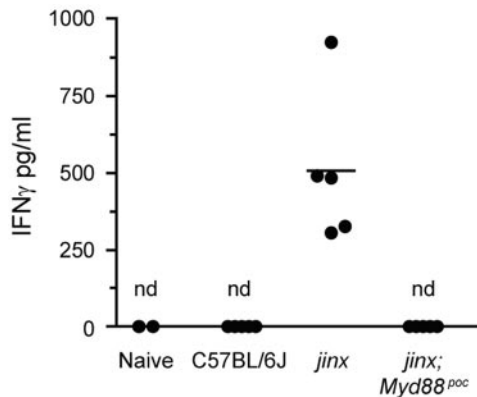
Figure 4. Systemic IFNγ 12 days after LCMV infection. Serum IFNγ was measured by ELISA. Each dot represents an individual mouse. Nd indicates not detected; *jinx*, *Unc13d$^{jinx/jinx}$* mice; *jinx;Myd88^{poc}*, *Unc13d$^{jinx/jinx}$;Myd88$^{poc/poc}$* mice.

Absence of MyD88 signaling in Unc13d$^{jinx/jinx}$;Myd88$^{poc/poc}$ mice prevents severe systemic inflammation and viral proliferation

Although they exhibited similar spleen size, complete blood counts, macrophage and CD8$^+$ T-cell numbers as wild-type mice, *Unc13d$^{jinx/jinx}$;Ifngr$^{-/-}$* mice produced overwhelming amounts of systemic IFNγ and TNF (supplemental Figure 2D-E). Serum IFNγ concentration was higher in *Unc13d$^{jinx/jinx}$;Myd88$^{poc/poc}$* double mutants than in wild-type controls on day 7 at the peak of the CD8$^+$ T-cell response (supplemental Figure 2F). However, 12 days after infection with LCMV, IFNγ was undetectable in *Unc13d$^{jinx/jinx}$;Myd88$^{poc/poc}$* mice (Figure 4) which additionally displayed only a moderate quantity of serum TNF (supplemental Figure 2E). These observations show that compared with deletion of the *Ifngr* gene, the *Myd88^{poc}* mutation mitigates systemic inflammation in a remarkably effective manner. Although IFNγ may play a major role in the activation of APCs by primed CD8$^+$ T cells, it has nonetheless nonredundant functions in controlling viral proliferation, and is therefore necessary for host survival.[14] LCMV tends to persist in kidneys,[23] and whereas *Unc13d$^{jinx/jinx}$;Ifngr$^{-/-}$* compound mutants failed to clear the virus from the kidney on day 12 after inoculation, no infectious particles were detected in *Unc13d$^{jinx/jinx}$;Myd88$^{poc/poc}$* mice (supplemental Figure 2G). Noteworthy, LCMV was controlled in blood and spleens of *Unc13d$^{jinx/jinx}$;Myd88$^{poc/poc}$* and *Unc13d$^{jinx/jinx}$;Ifngr$^{-/-}$* mice on day 12 (data not shown).

Finally, we performed histologic analysis to further investigate the dampening effects of MyD88 disruption on HLH-like symptoms in *Unc13d$^{jinx/jinx}$* mice. Sections from spleens revealed absent or disrupted B-cell follicles with infiltrations of activated lymphocytes and macrophages in LCMV-infected *Unc13d$^{jinx/jinx}$* mice.

Unc13d$^{jinx/jinx}$;Myd88$^{poc/poc}$ compound mutants, however, displayed distinct germinal centers, although their overall splenic architecture was to some extent less organized than in wild-type controls (Figure 5).

Discussion

We have shown that mutational inactivation of MyD88 prevents HLH-like disease in *Unc13d$^{jinx/jinx}$* mice by abrogating the positive feedback loop between CD8$^+$ T cells and APCs while permitting effective control of infection. Replicating virus was absent in *Unc13d$^{jinx/jinx}$;Myd88$^{poc/poc}$* mutants, in contrast with a large infectious reservoir in *Unc13d$^{jinx/jinx}$;Ifngr$^{-/-}$* kidneys (supplemental Figure 2G). This has important clinical implications toward a strategy to eliminate HLH immunopathology while maintaining antiviral immunity. Several mechanisms and effector cell types may account for the balance between immunopathology and protection observed in *Unc13d$^{jinx/jinx}$;Myd88$^{poc/poc}$* mice.

Besides TLR signaling, MyD88 mediates signaling from the IL-1 receptor family, which includes the IL-18 receptor expressed by CD8$^+$ T cells. In a LCMV model of neuropathogenesis, disruption of the IL-18 or IL-1 receptors did not affect CD4$^+$ T-cell response whereas disruption of MyD88 did.[24] IL-18 synergizes with IL-12 in maintaining IFNγ secretion by LCMV-specific CD8$^+$ T cells.[25-26] Furthermore, administration of an IL-18–blocking antibody to LCMV-infected perforin-deficient mice, which also exhibit HLH-like clinical features, did not improve the survival of treated animals.[17] Therefore abrogation of IL-18 signaling in CD8$^+$ T cells by compound homozygosity for the *Myd88^{poc}* mutation is unlikely to explain rescue of HLH-like disease in *Unc13d$^{jinx/jinx}$;Myd88$^{poc/poc}$* mice.

High levels of IL-1 are found in the serum of HLH-affected patients,[13] and the administration of IL-1 receptor antagonists seems beneficial for patients affected with the reactive form of HLH.[27] To determine the degree of involvement of IL-1 in the development of the HLH-like syndrome in response to LCMV infection, *Unc13d$^{jinx/jinx}$* mice were bred to IL-1R1–deficient mice. Disrupting IL-1R1 signaling neither decreased the maturation state of *Unc13d$^{jinx/jinx}$* macrophages (supplemental Figure 4A) nor restrained activation of *Unc13d$^{jinx/jinx}$* CD8$^+$ T cells in response to LCMV (supplemental Figure 4B-C), suggesting that the rescue of LCMV-infected *Unc13d$^{jinx/jinx}$* mice by concomitant MyD88-deficiency does not result from impairment of IL-1 receptor signaling.

Our data exclude a role for TNF and IL-1 for the progression of HLH-like disease in *Unc13d$^{jinx/jinx}$* mutants (supplemental Figures 2 and 4, respectively). Yet IL-6–producing macrophages have been
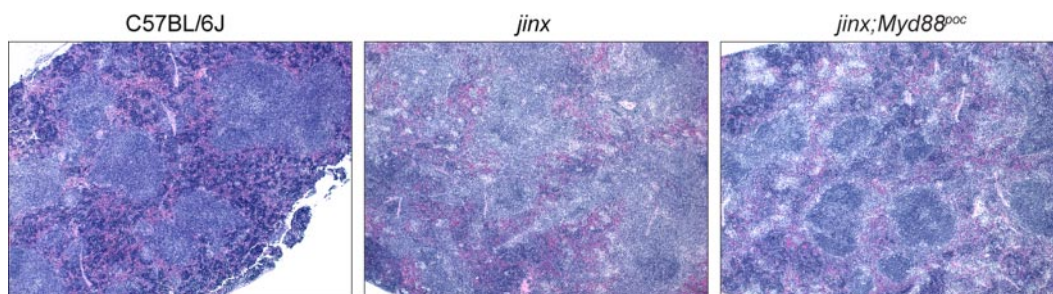

Figure 5. Histologic appearance of the spleen in LCMV-infected mutant and wild-type mice. Hematoxylin and eosin staining of sections of spleen from representative mutant and wild-type mice 12 days after LCMV infection. Images were acquired using an Olympus AX70 microscope equipped with a 4× UPlanFl objective with a numeric aperture of 0.13, a SPOT-RT slider camera and SPOT software Version 4.6. 40× magnification is displayed. *jinx* indicates *Unc13d$^{jinx/jinx}$* mice; *jinx;Myd88^{poc}*, *Unc13d$^{jinx/jinx}$;Myd88$^{poc/poc}$* mice.

identified in liver biopsies and blood of patients suffering from hemophagocytic syndrome[28-29] and significant amount of IL-6 was found in sera of mice displaying symptoms of HLH.[17] On LCMV infection, MyD88-deficiency results in reduced production of IL-6,[20,22] which has been suggested to support T-cell proliferation.[30] However, $Unc13d^{jinx/jinx};Myd88^{poc/poc}$ double mutants displayed serum IL-6 concentrations that were not significantly different from control C57BL/6J or $Unc13d^{jinx/jinx}$ mice on day 7 after LCMV infection (supplemental Figure 4D). These data suggest a minor role of this cytokine for the outcome of the T-cell response in our model.

The observation that mice with deficiencies in both TLR7 and TLR9 mount normal adaptive immune responses to LCMV supports a TLR-independent role of MyD88 for containing the infection.[20] Alternatively, sensors independent of MyD88 may be sufficient to induce normal adaptive responses to LCMV in MyD88-deficient mice. Recently, the endoplasmic RNA helicases RIG-I and MDA-5 were shown to bind LCMV-derived RNA and trigger type I IFN production.[31] Additional cell-intrinsic roles have also been ascribed to MyD88 as for instance its ability to regulate expression of effector immune responses in IFNγ-activated macrophages.[32] MyD88 has been shown to interact with IFNγR thereby transducing IFNγ-mediated signals that induce a pathway for the control of expression and stability of many short-lived mRNAs, including those encoding TNF and IFNγ-inducible protein 10 (IP-10).[33]

During LCMV infection, the priming of the immune response largely depends on LCMV-infected APCs.[34-35] It has been suggested that impaired type I IFN production by plasmacytoid dendritic cells is an underlying cause of reduced priming of endogenous T cells in $Myd88^{-/-}$ mice.[20] However, MyD88-deficient APCs were shown to be as efficient as wild-type APCs in priming infused, MyD88-competent T cells with a transgenic T-cell receptor specific for the immunodominant epitope of LCMV.[22] Furthermore, serum IFNβ remained undetectable in LCMV-infected mice on day 7, although substantial concentrations of IFNγ were measured (data not shown and supplemental Figure 2F), thereby further emphasizing the importance of IFNγ for the modulation of the T-cell response in $Unc13d^{jinx/jinx}$ mice.

Thus, a need for MyD88 in costimulation and antigen presentation leading to T-cell activation is unlikely to be a critical driver of HLH-like disease per se in LCMV-infected $Unc13d^{jinx/jinx}$ mice, at least during the acute stage of the infection. Yet, alteration of T-cell responses observed in the MyD88-deficient state might potentially result from a requirement for MyD88 within APCs.

MyD88 is expressed by T cells and required for their function. Therefore, $Unc13d^{jinx/jinx};Myd88^{poc/poc}$ CD8^{+} T cells might have an intrinsic defect imparted by the $Myd88$ mutation. In mice infected with LCMV, MyD88 signaling is necessary for sustained expansion of effector CD8^{+} T cells,[21-22] especially during systemic infection with the faster-replicating Traub strain of LCMV.[36] However MyD88 is not involved in early activation and proliferation of antigen-activated CD8^{+} T cells nor in the generation and maintenance of functional memory CD8^{+} T cells.[37] Considering the importance of IFNγ-producing CD8^{+} T cells for the development of LCMV-induced HLH disease,[17] it is plausible that disruption of MyD88 rescues the disease in $Unc13d^{jinx/jinx};Myd88^{poc/poc}$ mutants because fewer CD8^{+} T cells secreting less IFNγ are induced. This may, in turn, result in fewer APCs being activated, thereby restricting the stimulation of CD8^{+} T cells. In addition to such a quantitative effect on APC activation, the transcription of many IFNγ-dependent genes in macrophages also relies on MyD88,[32] which may further affect those macrophages that become activated in $Unc13d^{jinx/jinx};Myd88^{poc/poc}$ mice.

In summary, our data demonstrate no role for CD18, TNF, and IL-1R1 in the progression of HLH in our model. Furthermore, we identified MyD88 as a candidate drug target superior to IFNγ, given the unrestrained viral load resulting from a disruption of IFNγ signaling.

Authorship

Contribution: P.K. and K.C. conducted experiments and performed data analyses; D.P. conducted experiments, performed data analyses, and provided critical discussions; M.B.O. provided critical discussions; and P.K., K.C., and B.B. designed experiments and wrote the manuscript.

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