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Autophagy Genes Enhance Murine Gammaherpesvirus 68 Reactivation from Latency by Preventing Virus-Induced Systemic Inflammation

Graphical Abstract

Highlights
- Autophagy (Atg) genes in myeloid cells inhibit virus-triggered systemic inflammation
- Atg gene-regulated systemic inflammation inhibits herpesvirus reactivation
- Interferon-γ controls herpesvirus reactivation in the setting of Atg gene mutations

Authors
Sunmin Park, Michael D. Buck, Chandni Desai, ..., Tiffany A. Reese, Maxim N. Artyomov, Herbert W. Virgin

Correspondence
virgin@wustl.edu

In Brief
Park et al. report that canonical autophagy genes in myeloid cells regulate the level of systemic inflammation induced by chronic murine gammaherpesvirus infection. One consequence of increased systemic inflammation in the setting of autophagy-gene deficiency is the inhibition of reactivation of the herpesvirus from latently infected macrophages.
Autophagy Genes Enhance Murine Gammaherpesvirus 68 Reactivation from Latency by Preventing Virus-Induced Systemic Inflammation

Sunmin Park,1 Michael D. Buck,1 Chandni Desai,1 Xin Zhang,1 Ekaterina Loginicheva,1 Jennifer Martinez,2 Michael L. Freeman,3 Tatsuya Saitoh,4 Shizuo Akira,5 Jun-Lin Guan,6 You-Wen He,7 Marcia A. Blackman,8 Scott A. Handley,1 Beth Levine,9 Douglas R. Green,10 Tiffany A. Reese,11 Maxim N. Artyomov,1 and Herbert W. Virgin1,*

1Department of Pathology and Immunology, Washington University in St. Louis, St. Louis, MO 63110, USA
2Department of Immunology, St Jude Children’s Research Hospital, Memphis, TN 38105, USA
3Department of Immunology, Duke University Medical Center, Durham, NC 27710, USA
4Department of Cancer Biology, University of Cincinnati College of Medicine, Cincinnati, OH 45267, USA
5Department of Host Defense, Research Institute for Microbial Diseases, Osaka University, 3-1 Yamada-oka, Suita, Osaka 565-0871, Japan
6Department of Immunology, University of Cincinnati College of Medicine, Cincinnati, OH 45267, USA
7Department of Immunology, Duke University Medical Center, Durham, NC 27710, USA
8Trudeau Institute, Saranac Lake, NY 12983, USA
9Center for Autophagy Research, Department of Internal Medicine and Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA
10Department of Immunology, St Jude Children’s Research Hospital, Memphis, TN 38105, USA
11Department of Immunology and Microbiology, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA
*Correspondence: virgin@wustl.edu
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SUMMARY

Host genes that regulate systemic inflammation upon chronic viral infection are incompletely understood. Murine gammaherpesvirus 68 (MHV68) infection is characterized by latency in macrophages, and reactivation is inhibited by interferon-γ (IFN-γ). Using a lysozyme-M-cre (LysMcre) expression system, we show that deletion of autophagy-related (Atg) genes Fip200, beclin 1, Atg14, Atg16L1, Atg7, Atg3, and Atg5, in the myeloid compartment, inhibited MHV68 reactivation in macrophages. Atg5 deficiency did not alter reactivation from B cells, and effects on reactivation from macrophages were not explained by alterations in productive viral replication or the establishment of latency. Rather, chronic MHV68 infection triggered increased systemic inflammation, increased T cell production of IFN-γ, and an IFN-γ-induced transcriptional signature in macrophages from Atg gene-deficient mice. The Atg5-related reactivation defect was partially reversed by neutralization of IFN-γ. Thus Atg genes in myeloid cells dampen virus-induced systemic inflammation, creating an environment that fosters efficient MHV68 reactivation from latency.

INTRODUCTION

Herpesvirus infections are life-long due to the establishment of latency, a molecularly quiescent form of infection, and reactivation from latency to generate infectious virus (Speck and Ganem, 2010). Chronic human gammaherpesvirus infection with Epstein-Barr virus or Kaposi’s sarcoma-associated herpesvirus is associated with lymphoproliferative diseases (Cesarman et al., 1995; Chang et al., 1994; Nador et al., 1996; Young et al., 1989). Infection of mice with the related murine gammaherpesvirus 68 (MHV68, γHV68, MuHV4) also results in lymphoproliferative disease (Tarakanova et al., 2005, 2008). After resolution of acute productive infection, MHV68 persists in mice by establishing latency in peritoneal macrophages and splenic B cells independent of the route of infection (Flanio et al., 2000; Tibbetts et al., 2003; Weck et al., 1996, 1999a, 1999b). Interferon-γ (IFN-γ) inhibits, and IL-4 increases, reactivation from latently infected macrophages by regulating viral promoters for the essential immediate early gene 50 (Barton et al., 2011; Goodwin et al., 2010; Reese et al., 2014; Steed et al., 2006, 2007).

Macroautophagy (termed canonical autophagy herein) degrades cytoplasmic cargo captured within double membrane-bound autophagosomes, which fuse with lysosomes to generate autolysosomes (Levine et al., 2011). We refer to canonical autophagy to distinguish it from topologically distinct cellular processes that require certain Atg genes such as LC3-associated phagocytosis (LAP), secretion, and control of parasite and viral replication by IFN-γ (Bestbroer et al., 2013; Choi et al., 2014; DeSelm et al., 2011; Renault et al., 2012; Hwang et al., 2012; Martinez et al., 2015; Reggiori et al., 2010; Sanjuan et al., 2007; Zhao et al., 2008). Canonical autophagy involves activation of the ULK1 complex (ULK1-ATG13-FIP200-ATG101) and the class III phosphatidylinositol-3-OH kinase (PI3K) complex (VPS34-VPS15-Becn1-ATG14). Two ubiquitin-like protein conjugation systems conjugate LC3 family members to phosphatidylethanolamine and ATG12 to ATG5 (Weidberg et al., 2010, 2011) in reactions requiring ATG7 as an E1-like enzyme. ATG10, ATG5, and ATG16L1 are involved in generating ATG5-ATG12 conjugates while ATG3 and ATG4 are involved in LC3 lipidation. SNAREs
mediate the fusion of autophagosomes and lysosomes (Diao et al., 2015; Itakura et al., 2012; Nair et al., 2011). Atg genes regulate innate and adaptive immunity (Dupont et al., 2011; Lupfer et al., 2013; Nakahira et al., 2011; Saitoh et al., 2008; Shi et al., 2012) and participate in LAP (Hennault et al., 2012; Huang et al., 2009; Martinez et al., 2011, 2015; Sanjuan et al., 2007). Auto-

phagy and Atg gene-dependent cellular functions control intracel-

2ular pathogens such as viruses (Hwang et al., 2012; Orvedahl et al., 2007, 2010) and are countered by herpesvirus virulence pro-

2teins (E et al., 2009; Lee and Sugden, 2008; Lee et al., 2009; Leidal et al., 2012; Liang et al., 2013; Loh et al., 2005; Orvedahl et al., 2007; Takahashi et al., 2009; Yordy et al., 2012).

Since Atg genes and canonical autophagy control both viral replication in a cell-intrinsic manner and inflammatory processes that might influence viral infection in a cell-extrinsic manner, we defined the role of Atg genes during MHV68 infection. We found that myeloid cell expression of multiple Atg genes was required for efficient MHV68 reactivation from murine macrophages, but not for viral replication or establishment of latency. This function of Atg genes was through inhibition of virus-triggered systemic inflammation rather than effects intrinsic to infected cells. Some Atg genes required for efficient reactivation are not required for LAP, arguing that canonical autophagy inhibited virus-triggered systemic inflammation. Thus, Atg genes prevent excessive sys-

temic inflammation during chronic herpesvirus infection. Interesting, in a companion paper we found that certain Atg genes act in myeloid cells to prevent lung inflammation and thereby foster lethal influenza virus infection (Lu et al., 2016). Together these studies suggest that a common role for Atg genes in myeloid cells is to prevent tissue-specific and virus-induced inflammation and that this can have significant effects on infectious disease.

**RESULTS**

**Multiple Autophagy Genes Promote MHV68 Reactivation from Macrophages**

To investigate whether Atg genes regulate chronic MHV68 infection, we used mice with Atg genes flanked by loxP sites, Atg<sup>xox/fox</sup> (Atg<sup>xox</sup>), bred to mice expressing Cre recombinase under the control of the lysozyme-M (LysMcre) promoter (Claussen et al., 1999; Hwang et al., 2012; Jakubzick et al., 2008). Several studies have demonstrated canonical autophagy-independent functions of Atg genes (Choi et al., 2014; DeSelm et al., 2011; Hennault et al., 2012; Hwang et al., 2012; Sanjuan et al., 2007; Zhao et al., 2008). We selected Atg genes involved in various stages of autophagy, recognizing that in macrophages beclin 1, Atg5, Atg7, Atg16l1, and Atg3 are required for both canonical autophagy and LAP, while Fip200 and Atg14 are not required for LAP (Martinez et al., 2015).

We assessed reactivation from latency 42 days after MHV68 infection, when productive infection has been cleared, using a limiting dilution reactivation assay (LDA) in which cell populations containing latently infected cells are explanted onto monolayers of murine embryonic fibroblasts (MEFs) for 2–3 weeks and the cells are assayed in parallel by disrupting explanted cells in a manner that prevents reactivation but that does not inactivate infectious virus.

MHV68 reactivation from peritoneal exudate cells (PECs) is overwhelmingly from latently infected macrophages (Weck et al., 1999b). MHV68 reactivated inefficiently from peritoneal macrophages from mice lacking Fip200, beclin 1, or Atg14 in LysM-expressing cells (Figure 1A). LysM-specific deletion of genes involved in the ubiquitin-like conjugations systems of autophagy including Atg5, Atg7, Atg16l1, and Atg3 resulted in a similar defect in MHV68 reactivation (Figure 1B). The difference between mice mutated for Atg3 versus other genes is

**Figure 1. Multiple Autophagy Genes Promote MHV68 Reactivation from Macrophages**

(A–D) Fip200<sup>LysMcre</sup>, beclin 1<sup>LysMcre</sup>, and Atg14<sup>LysMcre</sup> mice (A); Atg7<sup>LysMcre</sup>, LysMcre, Atg16l1<sup>LysMcre</sup>, LysMcre, Atg5<sup>LysMcre</sup>, and Atg3<sup>LysMcre</sup> mice (B); Atg4b<sup>−/−</sup> (C); and p62<sup>−/−</sup> (D) and wild-type littermates were infected with 1 × 10<sup>6</sup> PFU MHV68 intraperitoneally (i.p.) for 42 days to determine frequency of virus reactivation in peritoneal exudate cells (PECs) during latent infection using LDA analysis. Data points indicate the percentage of wells that were positive for cytopathic effect (CPE) on a mouse embryonic fibroblast (MEF) monolayer. All LDA experiments are n = 3 or 4 with 3–5 mice pooled per experiment. Data are the mean ± SEM and p values were obtained by paired t test over all dilutions. Only significant comparisons are indicated. *p < 0.05, **p < 0.01, ***p < 0.005, ****p < 0.001.
likely related to inefficient deletion of this gene in these lineages (Choi et al., 2014). Cells from Atg4b<sup>-/-</sup> mice reactivated normally (Figure 1C). There was no reactivation from splenocytes at this time point (Figure S1A and data not shown), and pre-formed infectious virus did not contribute to observed reactivation (Figure S1B). p62, an autophagy adaptor responsible for targeting Sindbis virus capsids to the autophagosome (Orvedahl et al., 2010), did not regulate MHV68 reactivation (Figure 1D). Thus, multiple Atg genes, but not p62, promote the MHV68 reactivation from latently infected macrophages. For subsequent studies, we focused on MHV68 reactivation using mice lacking Atg5.

**Autophagy Genes Control MHV68 Reactivation from Macrophages but Not from B Cells**

Since MHV68 establishes latency in splenic B cells, we evaluated MHV68 reactivation in mice lacking Atg5 in CD19-expressing cells (Atg5<sup>f/f</sup>-CD19cre) (Conway et al., 2013; Miller et al., 2008; Pengo et al., 2013) at 16 days after infection, a time when reactivation from explanted B cells is robust enough to be quantified. MHV68 reactivated less efficiently from peritoneal macrophages from Atg5<sup>f/f</sup>-LysMcre mice at this time point, but reactivation from splenic B cells was identical in Atg5<sup>f/f</sup> and Atg5<sup>f/f</sup>-CD19cre mice (Figure 2). Thus, Atg5 deficiency does not control MHV68 reactivation in B cells.

**Atg5 Is Not Required for Viral Replication or Establishment of Latent Infection**

Defective reactivation could be due to a failure of MHV68 to replicate in Atg5-deficient macrophages or to establish or maintain latent infection. However, viral replication was normal in bone marrow-derived macrophages (BMDMs) (Figure 3A) or PECs (Figure 3B) from Atg5<sup>f/f</sup>-LysMcre mice, and there was no Atg5-associated defect in replication in vivo as measured by plaque assay (Figure 3C) or light emission in mice infected with luciferase-expressing MHV68 (M3FL) (Hwang et al., 2008; Reese et al., 2014) (Figure 3D). Further, the frequency of virus-positive PECs during chronic infection was not affected by Atg5 mutation as measured by limiting dilution nested PCR targeting MHV68 viral orf72 (Figure 3E) (Weck et al., 1999a, 1999b).

Thus decreased reactivation was not due to a role for Atg5 in viral replication or latency establishment.

We next determined whether there was a paracrine factor from Atg5<sup>f/f</sup>-LysMcre PECs that inhibited reactivation by comparing virus reactivation in PECs from Atg5<sup>f/f</sup> and Atg5<sup>f/f</sup>-LysMcre mice to reactivation from a 1:1 mixture of these cell populations (Figure 3F). In the 1:1 mixture, we observed greater than the 2-fold decrease expected if changes in reactivation were merely due to the dilution of Atg5<sup>f/f</sup> cells by reactivation-defective Atg5<sup>f/f</sup>-LysMcre PECs. This finding could be explained by the secretion of a paracrine factor, likely of immune origin, capable of blocking viral reactivation by reactivation-defective cell populations.

**Atg5 Deficiency Increases Virus-Induced IFN-γ-Dependent Macrophage Activation**

We hypothesized that the immune response to MHV68 in Atg5<sup>f/f</sup>-LysMcre mice might differ from that in wild-type mice. The frequency of latently infected macrophages was ca. 1/1,000 PECs (Figure 3E), indicating that changes in overall cell populations would be due to systemic effects of Atg gene deficiency rather than effects intrinsic to these rare infected cells. As expected, MHV68 infection increased inflammatory monocytes regardless of genotype (Figure S2A) (Barton et al., 2007). However, neutrophils were increased selectively in latently infected Atg5<sup>f/f</sup>-LysMcre mice, consistent with a recent report of increased proliferation of peripheral neutrophils in Atg5<sup>f/f</sup>-LysMcre mice (Rozman et al., 2015).

We next assessed the steady-state level of transcripts in peritoneal macrophages during chronic MHV68 infection using RNA-seq. Gene set enrichment analysis demonstrated changes in transcription specific to macrophages from MHV68-infected
Increased T Cell-Derived IFN-γ Inhibits MHV68 Reactivation in Atg5f/f-LysMcre Mice

The gene expression data suggested a role for IFN-γ in systemic inflammation, a hypothesis supported by the observation that MHV68-infected Atg5f/f-LysMcre mice exhibited increased serum levels of IFN-γ (Figure 5A). Explanted PECs from these mice secreted increased amounts of IFN-γ (Figure 5B), indicating that a peritoneal cell type was producing increased IFN-γ. To determine the source of IFN-γ, we analyzed IFN-γ-producing cell types including NK cells and CD4 and CD8 T cells. Total numbers of these cells were identical between Atg5f/f and Atg5f/f-LysMcre mice (Figure 6A). Depletion of NK cells throughout infection in Atg5f/f and Atg5f/f-LysMcre mice did not change virus reactivation (Figure S3A). We observed an increase in IFN-γ-positive peritoneal CD4 T cells in latently infected Atg5f/f-LysMcre mice (Figure 6B). Depletion of CD4 and CD8 T cells decreased IFN-γ secretion by explanted PECs, suggesting that T cells were a major source of IFN-γ during chronic MHV68 infection of Atg5f/f-LysMcre mice (Figure 6C).

We did not find increased secretion of IL-12p70 or IL-18 or changes in cell surface expression of CD80, CD86, or MHC class II in macrophages from Atg5f/f and Atg5f/f-LysMcre mice that might explain these observations (Figures S3B and S3C). We adoptively transferred transgenic CD4 T cells expressing a T cell receptor (TCR) specific for MHV68 glycoprotein gp150 (Freeman et al., 2011) and CD8 T cells expressing a TCR specific for ovalbumin (Loh et al., 2012) into Atg5f/f and Atg5f/f-LysMcre mice followed by infection with MHV68 expressing ovalbumin.
PECs were harvested 28 days later and stimulated with specific (gp150 or ovalbumin) and control (influenza NP366-374) peptides (Figures S3D and S3E). Virus-specific CD4 and CD8 peritoneal T cells expressed IFN-γ after antigen stimulation similarly regardless of whether they came from Atg5f/f or Atg5f/f-LysMcre mice, indicating that enhanced IFN-γ production is due to an in vivo effect not duplicated in ex vivo T cell stimulation assays.

IFN-γ Neutralization Rescues MHV68 Reactivation in PECs from Atg5f/f-LysMcre Mice

Data on elevated IFN-γ levels were of interest because this cytokine inhibits MHV68 reactivation from latency (Goodwin et al., 2010; Steed et al., 2006, 2007) with minimal effects on acute replication of MHV68 (Dutia et al., 1997; Sarawar et al., 1997; Tibbetts et al., 2002; Weck et al., 1997) or establishment of latency (Sarawar et al., 1997; Steed et al., 2006; Tibbetts et al., 2002). IFN-γ neutralization at the time of explant partially restored reactivation in PECs from Atg5f/f-LysMcre mice (Figure S4A), but as the reactivation curve did not cross the Poisson distribution line, it was not possible to calculate the statistical significance for this reproducible effect (Figure S4B). There was no effect of anti-IFN-γ treatment upon explant on reactivation from PECs from Atg5f/f mice. When IFN-γ was neutralized throughout the course of MHV68 infection in Atg5f/f-LysMcre in vivo and in explant cultures, we observed increased reactivation in macrophages from Atg5f/f-LysMcre mice to the level observed in macrophages from untreated Atg5f/f mice (Figure 7), with no preformed virus detected in anti-IFN-γ treated groups (Figure S4C). This procedure also increased reactivation of macrophages from Atg5f/f mice. Differences in virus reactivation remained between Atg5f/f and Atg5f/f-LysMcre mice after neutralization of IFN-γ, suggesting that this cytokine may not be the sole contributor to decreased reactivation observed in Atg gene-deficient mice. Thus IFN-γ expression in vivo plays a role in limiting reactivation of MHV68 from cells derived from an autophagy-deficient environment.

DISCUSSION

We report that deletion of Atg genes in LysM-expressing cells resulted in decreased efficiency of MHV68 reactivation from latent infection and increased virus-triggered systemic inflammation, including high circulating IFN-γ levels and expression of TNF and IFN-γ pathway genes in macrophages. IFN-γ is likely an important link between systemic inflammation and decreased reactivation from latency, as this cytokine potently inhibits...
The involvement of all of these autophagy, a process requiring all of the multiple isoforms of Atg4 genes tested (Lev- ene et al., 2011), and neutralization of IFN-γ increased viral reactivation in macrophages from mice lacking Atg5 in LysM-expressing cells. Thus, Atg genes in myeloid cells can influence the course of chronic viral infection through effects on the overall inflammatory state of the host and are responsible for limiting systemic inflammation during chronic herpesvirus infection.

Role of Atg Genes in Myeloid Cells in Control of Herpesvirus Reactivation
Deleting Atg genes including Atg5, Atg16L1, Atg7, Atg3, Atg14, beclin 1, and Fip200 in myeloid cells resulted in inhibition of MHV68 reactivation from latently infected macrophages. However, mice lacking Atg4b had normal frequencies of reactivation. This discrepancy may be attributed to the existence of multiple isoforms of Atg4 (Choi et al., 2014; Mariño et al., 2010). The involvement of all of these Atg genes in control of reactivation is most consistent with a requirement for canonical autophagy, a process requiring all of the Atg genes tested (Levine et al., 2011; Martinez et al., 2015) in control of chronic virus infection. The involvement of Atg14 and Fip200 is informative, as these genes are not required for LAP (Martinez et al., 2015). This differs from other situations in which canonical autophagy-independent functions of Atg genes control pathogen infection (Choi et al., 2014; Hwang et al., 2012; Reggiori et al., 2010; Zhao et al., 2008).

Cell-Intrinsic Versus Cell-Extrinsic Effects of Atg Genes on Viral Infection
IFN-γ inhibits murine norovirus growth in macrophages in a manner dependent on Atg5, Atg7, and Atg16L1, but not on Atg14 or the degradative function of canonical autophagy (Hwang et al., 2012). Atg genes can also have pro-viral actions in infected cells, such as in the release of picornaviruses from infected cells (Alirezaei et al., 2012; Taylor and Kirkegaard, 2008) and the replication of Dengue virus, coronavirus, and hepatitis C virus (Dreux et al., 2009; Heaton et al., 2010; Reggiori et al., 2010). In these situations Atg genes act directly in the infected cell. In contrast, we found no evidence for a cell-intrinsic role for Atg5 in MHV68 replication. Instead, Atg gene deficiency enhanced virus-triggered systemic immune activation during chronic infection, which resulted in decreased reactivation from latency. These data reveal inhibition of systemic inflammation as an additional mechanism through which Atg genes regulate viral infection.

MHV68 encodes a viral Bcl-2 (v-Bcl-2) protein reported to inhibit both autophagy and apoptosis. Analysis of MHV68 v-Bcl-2 mutants in BALB/c mice supports a role for v-Bcl-2-mediated inhibition of autophagy in maintenance of latency, while the anti-apoptotic function fosters viral reactivation (E et al., 2009). However, in C57BL/6J mice, full deletion of v-Bcl-2 only modestly inhibits viral reactivation from latency without changing the frequency of latently infected cells (Gangappa et al., 2002; Loh et al., 2005). Using C57BL/6J mice, we observed profound effects of deletion of Atg genes in the myeloid lineage on reactivation but not establishment or maintenance of viral latency. Since deletion of v-Bcl-2 (which should increase autophagy in infected macrophages) and the deletion of Atg genes in myeloid cells (which inhibits autophagy in both infected and uninfected cells) both result in decreased reactivation, the data strongly support the concept that the dominant effects observed here are due to Atg gene effects outside of the infected cell. This further supports our conclusion that regulation of virus-induced systemic inflammation is an important role for Atg genes.

Role of Autophagy and Atg Genes in Controlling Inflammation
Numerous studies have identified Atg genes and canonical autophagy in macrophages as key regulators of inflammation (Abdel Fattah et al., 2015; Deretic et al., 2015; Kanayama et al., 2015; Levine et al., 2011). While we measured events that occur in macrophages such as gene expression and viral reactivation, LysMCre-mediated gene deletion occurs in multiple cell types including neutrophils and some dendritic cells (Clausen et al., 1999; Jakubzik et al., 2008). Therefore, the effects on macrophage-dependent assays presented here could be due to effects of Atg genes in either macrophages or other LysM-expressing cell types that regulate inflammation triggered by chronic virus infection.

We found that peritoneal macrophages from uninfected Atg5f/f-LysMcre mice exhibited a significant upregulation of IFN-γ response pathway genes, an effect likely due to increased MHV68 infection-driven IFN-γ expression in CD4 T cells. Macrophages from uninfected Atg5f/f-LysMcre mice were hyper-responsive to IFN-γ treatment. Interestingly, Atg5-deficient BMDMs have largely normal responses to IFN-γ (Hwang et al., 2012; Zhao et al., 2008), strongly indicating that events in vivo are triggered Atg gene-controlled inflammation, even without the stimulus of chronic virus infection. These findings suggest that Atg genes are required to limit basal

A Figure 5. Elevated IFN-γ in Atg5f/f-LysMcre Mice Drives Macrophage Activation
(A) Serum levels of IFN-γ were measured by Luminex xMAP technology 42 days after infection. Each data point represents one mouse. Data were analyzed by one-way ANOVA with Kruskal-Wallis test with multiple comparisons. *p < 0.05, ***p < 0.005.
(B) 40,000 PECs pooled from three Atg5f/f mice or Atg5f/f-LysMcre mice were plated onto a monolayer of MEFs for 24 hr in a 96-well plate. IFN-γ levels in the supernatants were measured by a sandwich ELISA. The data are four biological replicate experiments with 24 technical replicate wells per sample. Each data point represents a technical replicate and was analyzed by the non-parametric Mann-Whitney U test. ***p < 0.005.
inflammation and that a trigger such as chronic viral infection can amplify the abnormal basal inflammation observed when myeloid cells lack \textit{Atg} genes. The fact that basal lung inflammation is also inhibited by \textit{Atg} genes (Lu et al., 2016), and that the lung inflammation that results from deletion of these genes in myeloid cells prevents lethal influenza virus infection, underlines the general role of \textit{Atg} genes in regulating infection through effects on inflammation.

**Implications for Disease and the Biology of Chronic Viral Infections**

We and others have documented the consequences of chronic viral infections and the mammalian virome in shaping immune responses to secondary infections and other challenges (Barton et al., 2007; MacDuff et al., 2015; Osborne et al., 2014; Reese et al., 2014; Stelekati et al., 2014; Virgin, 2014). For example, chronic MHV68 infection induces prolonged low-level production of IFN-\(\gamma\) and activation of macrophages in normal mice, resulting in symbiotic resistance to bacterial infection (Barton et al., 2007). Data presented here indicate that this process is enhanced in mice lacking \textit{Atg5} or \textit{Atg16l1} in myeloid cells. Our data suggest that autophagy plays a key role in limiting inflammation caused by common chronic viral infections, thereby protecting the host from diseases worsened by chronic inflammation. This may be significant, as most or all humans carry multiple chronic virus infections, and systemic inflammation is linked to many human diseases including cancer, diabetes, cardiovascular disease, and autoimmunity (Virgin, 2014). It is important to note that all experiments presented here are loss-of-function in design and therefore do not demonstrate that induction of autophagy would limit basal or virus-induced inflammation. However, our data support testing the hypothesis that basal and virus-triggered inflammation may be lessened by induction of autophagy.

One could speculate that chronic herpesvirus infection sets the stage, in an \textit{Atg} gene-dependent manner, for protective effects of innate or adaptive immunity and harmful effects of inflammation or autoimmunity in persons with specific genetic constitutions. For example, chronic MHV68 infection complemented genetic immunodeficiency due to mutation of \textit{Holl-1}, but did so at the cost of increased systemic inflammation (Boisson et al., 2012; MacDuff et al., 2015). Studies presented here combined with prior findings support the “virus plus host gene” concept that significant physiologic consequences develop when chronic virus infection intersects with host genetic susceptibility (Cadwell et al., 2008, 2010; Kernbauer et al., 2014; Virgin, 2014).

**EXPERIMENTAL PROCEDURES**

**Mice**

\textit{Atg5}\(^{fl/fl}\), \textit{Atg5}\(^{fl/fl}\)-\textit{LysMcre}, and \textit{Atg5}\(^{fl/fl}\)-\textit{CD19cre} mice were generated as described previously in an enhanced barrier facility (Miller et al., 2008; Zhao et al., 2008). \textit{Atg16l1}\(^{fl/fl}\)-\textit{LysMcre} (Hwang et al., 2012), \textit{Atg3}\(^{fl/fl}\)-\textit{LysMcre} (DeSelm et al., 2011), \textit{Atg14}\(^{fl/fl}\)-\textit{LysMcre} (Choi et al., 2014), \textit{Atg3}\(^{fl/fl}\)-\textit{LysMcre} (Choi et al., 2014), \textit{beclin 1}\(^{fl/fl}\)-\textit{LysMcre} (Sanjuan et al., 2007), and \textit{Fip200}\(^{fl/fl}\)-\textit{LysMcre} (Gan et al., 2006) were generated in the same way as \textit{Atg5}\(^{fl/fl}\)-\textit{LysMcre} (Komatsu et al., 2007) and \textit{Atg5b}\(^{fl/fl}\) (Manfio et al., 2010) mice were described elsewhere. All mice used for experimental procedures were 8–10 weeks of age.
MHV68 infection for 28 days in Atg5f/f and sex-matched littermates. Unless specified, all mice were infected with IFN-γ. Sera from naive or infected mice were collected by cardiac puncture. Cytokine Measurements by ELISA or Multiplex Cytokine Arrays.

Depletion of IFN-γ, NK, and T Cells In Vivo
For depletion of IFN-γ, clone H22 was used at 250 μg/mouse, injected i.p. every 7 days. Control hamster IgG (P75) was used for control groups. PECs were isolated in the presence of 10 μg/ml antibody and plated in the presence of these antibodies for evaluation of reactivation. NK cells were depleted with anti-NK1.1 (clone PK136) at 200 μg/mouse injected i.p. every 5 days beginning at 2 days prior to infection and continued until sacrifice. Mouse anti-rabbit isotype control was used for control groups. Reactivation was measured in the presence of 10 μg/ml antibody. To deplete T cells, CD4 (clone YTS 101.5) or CD8 (clone H35 17.2) depleting antibodies were injected 500 μg/mouse i.p. every other day (Chachu et al., 2008), 2 days before sacrifice. Isotype-matched control (SFR-DR5, IgG2b) was administered to control groups. Additional experimental procedures are available in the Supplemental Information.

ACCESSION NUMBERS
RNA-seq numbers are available at the European Nucleotide Archive: PRJEB10074.

SUPPLEMENTAL INFORMATION
Supplemental Information includes Supplemental Experimental Procedures and four figures and can be found with this article online at http://dx.doi.org/10.1016/j.chom.2015.12.010.

AUTHOR CONTRIBUTIONS
H.W.V., S.P., D.R.G., B.L., and T.A.R. conceived experiments. S.P. and H.W.V. wrote the manuscript. All authors reviewed the manuscript. S.P. performed all experiments. M.N.A., M.D.B., C.D., E.L., and X.Z. performed specific experiments. M.L.F., T.S., S.A., J.-L.G., J.M., Y.-W.H., and M.A.B. provided reagents. B.L., D.R.G., S.A.H., M.N.A., and H.W.V. provided expertise and feedback.

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