Immunity
Article

T Helper 2 Cytokines Inhibit Autophagic Control of Intracellular *Mycobacterium tuberculosis*

James Harris,[1,4,5] Sergio A. De Haro,[1,5] Sharon S. Master,[1] Joseph Keane,[2] Esteban A. Roberts,[1] Monica Delgado,[1] and Vojo Deretic[1,3,*]
[1]Department of Molecular Genetics and Microbiology, University of New Mexico Health Sciences Center, University of New Mexico School of Medicine, 915 Camino de Salud NE, Albuquerque, NM 87131, USA
[2]St. James's Hospital and Trinity College Dublin, Dublin 8, Ireland
[3]Department of Cell Biology and Physiology, University of New Mexico School of Medicine, Albuquerque, NM 87131, USA
[4]Present address: Trinity Centre for Health Sciences, Trinity College Dublin and St. James's Hospital, Dublin 8, Ireland.
[5]These authors contributed equally to this work.
*Correspondence: vderetic@salud.unm.edu
DOI 10.1016/j.immuni.2007.07.022

SUMMARY

Autophagy is a recently recognized immune effector mechanism against intracellular pathogens. The role of autophagy in innate immunity has been well established, but the extent of its regulation by the adaptive immune response is less well understood. The T helper 1 (Th1) cell cytokine IFN-γ induces autophagy in macrophages to eliminate *Mycobacterium tuberculosis*. Here, we report that Th2 cytokines affect autophagy in macrophages and their ability to control intracellular *M. tuberculosis*. IL-4 and IL-13 abrogated autophagy and autophagy-mediated killing of intracellular mycobacteria in murine and human macrophages. Inhibition of starvation-induced autophagy by IL-4 and IL-13 was dependent on Akt signaling, whereas the inhibition of IFN-γ-induced autophagy was Akt independent and signal transducer and activator of transcription 6 (STAT6) dependent. These findings establish a mechanism through which Th1-Th2 polarization differentially affects the immune control of intracellular pathogens.

INTRODUCTION

Autophagy is a fundamental homeostatic mechanism in which cells sequester discrete portions of the cytoplasm into an autophagosome, a specialized vacuole with a double membrane, which in turn delivers them to lysosomes for degradation (Levine, 2005; Shintani and Klionsky, 2004). This process removes damaged or surplus organelles such as leaky mitochondria and excess peroxisomes and, by degrading long-lived cytoplasmic macromolecules during periods of starvation, promotes cell survival (Kuma et al., 2004). Autophagy has recently been shown to play a role in innate immunity against intracellular pathogens, including Epstein-Barr Virus (Paludan et al., 2005), *Shigella flexneri* (Ogawa et al., 2005), *Salmonella typhimurium* (Birmingham et al., 2006), *Toxoplasma gondii* (Ling et al., 2006), and *Mycobacterium tuberculosis* (Gutierrez et al., 2004). In addition, autophagy has been implicated in adaptive immunity, playing a role in endogenous antigen presentation (Dengjel et al., 2005; Deretic, 2005; Paludan et al., 2005; Schmid et al., 2006b).

Autophagy can be induced pharmacologically with rapamycin, which inhibits TOR, a conserved Ser and Thr kinase that regulates cell growth and metabolism in response to growth factors, energy inputs, and nutritional demands (Wullschleger et al., 2006). TOR integrates various inputs; its activation stimulates anabolic processes and biomass production, whereas its inhibition enhances catabolic processes, including autophagy. A classical example of this system in action is demonstrated by amino acid starvation, which leads to inhibition of TOR and induction of autophagy. Conversely, TOR can be activated by growth factors via the Akt (also known as PKB) pathway, resulting in the inhibition of autophagy (Wullschleger et al., 2006). In some cases, withdrawal of growth factors is sufficient for induction of autophagy, even in the presence of adequate nutrients. For example, removal of IL-3 from cultures of an IL-3-dependent hemopoietic cell line that also lacks the apoptotic regulators Bax and Bak has been shown to induce autophagy, and if left unchecked, the cells eventually die (Lum et al., 2005). However, the cells die more rapidly when autophagy is blocked, suggesting that autophagy is a survival mechanism in these cells (Lum et al., 2005). Autophagy can be modulated by cytokines and other immunological signals (Andrade et al., 2006; Djavaheri-Mergny et al., 2006; Singh et al., 2006). For example, TNF-α can induce autophagy in Ewing sarcoma cells in the absence of NF-κB activation (Djavaheri-Mergny et al., 2006), whereas in macrophages and other cells, IFN-γ, a classical T helper 1 (Th1) cell cytokine and a critical antituberculosis immune mediator, induces or augments autophagy (Gutierrez et al., 2004; Inbal et al., 2002; Pyo et al., 2005). Moreover, the protective role of IFN-γ against mycobacteria has been associated with autophagy (Gutierrez et al., 2004; Singh et al.,

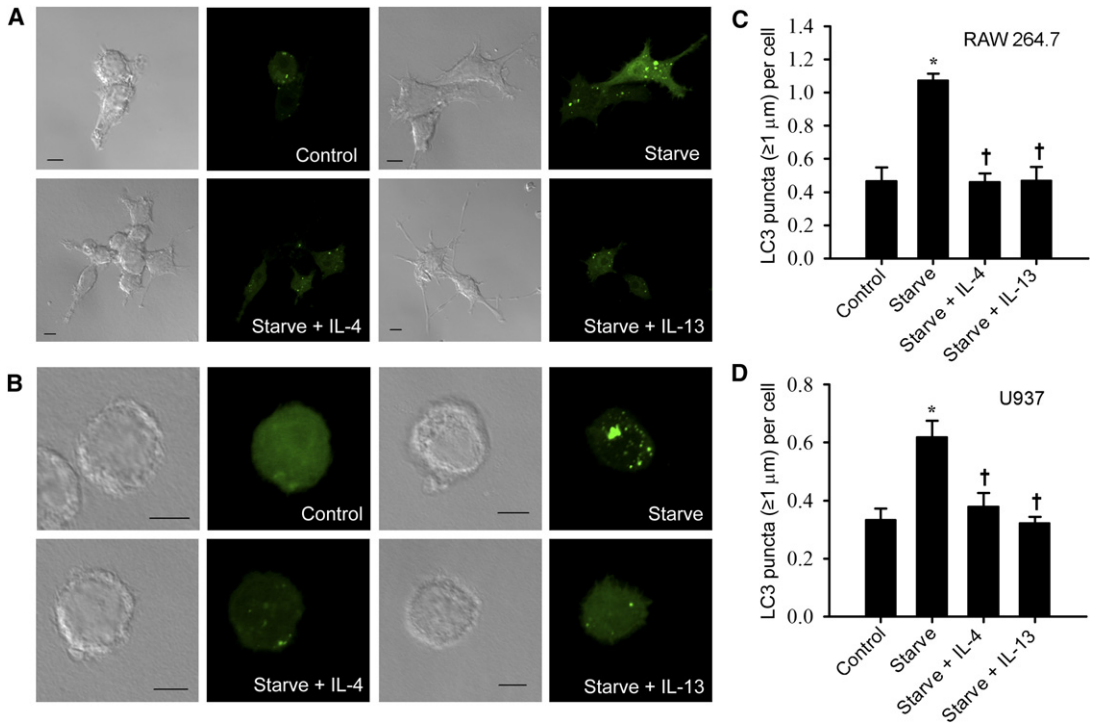

Figure 1. Inhibition of Starvation-Induced Autophagy by IL-4 and IL-13

(A) Murine RAW264.7 or (B) human U937 cells were transiently transfected with pEGFP-LC3, amino acid- and serum-starved for 2 hr with or without IL-4 or IL-13 (30 ng/ml), and analyzed by confocal microscopy. The number of large ($\geq$1 μm) LC3 puncta per cell were quantified (C and D). Data are presented as means ± SEM; *$p < 0.05$ and †$p \geq 0.05$; n = 3. Scale bars represent 5 μm.

2006), indicating that this process is an important effector mechanism of the Th1 response.

The two classical Th2 cytokines, IL-4 and IL-13, signal through the receptor IL-4Rα, which forms a heterodimer either with the gamma common (γc) chain (for IL-4) or the IL-13Rα1 (for IL-13) (Nelms et al., 1999). In addition, IL-13 can signal through the high-affinity IL-13Rα2, initially thought to be a decoy, nonsignaling receptor (Donaldson et al., 1998; Fichtner-Feigl et al., 2006; Kawakami et al., 2001; Zhang et al., 1997). Ligation of the IL-4 and IL-13 receptor complexes results in signaling via the insulin receptor substrate (IRS)-1 and 2 and STAT-6 pathways (Nelms et al., 1999). Although STAT-6 is involved in IL-4- and IL-13-induced gene expression, IRS-1 and 2 signaling activates the type I phosphatidylinositol 3-kinase (PI3K) pathway and subsequently the Akt pathway. On the basis of the latter signaling pathway, we hypothesized that IL-4 and IL-13 would inhibit the autophagy-induced killing of mycobacteria by macrophages. Here, we showed that IL-4 and IL-13 inhibited autophagy induced by either amino acid starvation or by IFN-γ in murine and human macrophages but that different signaling pathways were used to suppress starvation-induced versus IFN-γ-induced autophagy. This IL-4 and IL-13 action specifically inhibited the transfer of mycobacteria into lysosomal compartments and enhanced mycobacterial survival within infected macrophages by inhibiting autophagy.

RESULTS

IL-4 and IL-13 Inhibit Starvation-Induced Autophagy

Induction of autophagy in the murine RAW264.7 and human U937 monocyte or macrophage cell lines was monitored with morphometric analysis after the formation of pEGFP-LC3-labeled (Kabeya et al., 2000) autophagosomes ($\geq$1 μm). LC3 is the mammalian equivalent of yeast Atg8, a specific marker that translocates from the cytosol to autophagosomal membranes (Kabeya et al., 2000). Amino acid starvation resulted in a significant increase in the number of pEGFP-LC3$^+$ puncta per cell (Figure 1). Translocation of cytosolic LC3 to autophagosomal organelles was also detected by 4D live confocal microscopy (Chua and Deretic, 2004; Kyei et al., 2006; Roberts et al., 2006) in macrophages transfected with tdTomato-LC3 (Bjorkoy et al., 2005) (Movie SM1 in the Supplemental Data available online). Amino acid-starvation-induced formation of LC3 puncta was abrogated with the addition of 3-MA (Figure S1), a classical inhibitor of autophagy (Blommaart et al., 1997). After ascertaining the expression of IL-4Rα by flow cytometry (Figure S2), IL-4 and IL-13 were added to macrophages induced for autophagy by amino acid starvation. Either one of the Th2 cytokines tested abrogated autophagy, as shown by inhibition of pEGFP-LC3$^+$ puncta formation in both RAW and U937 cells

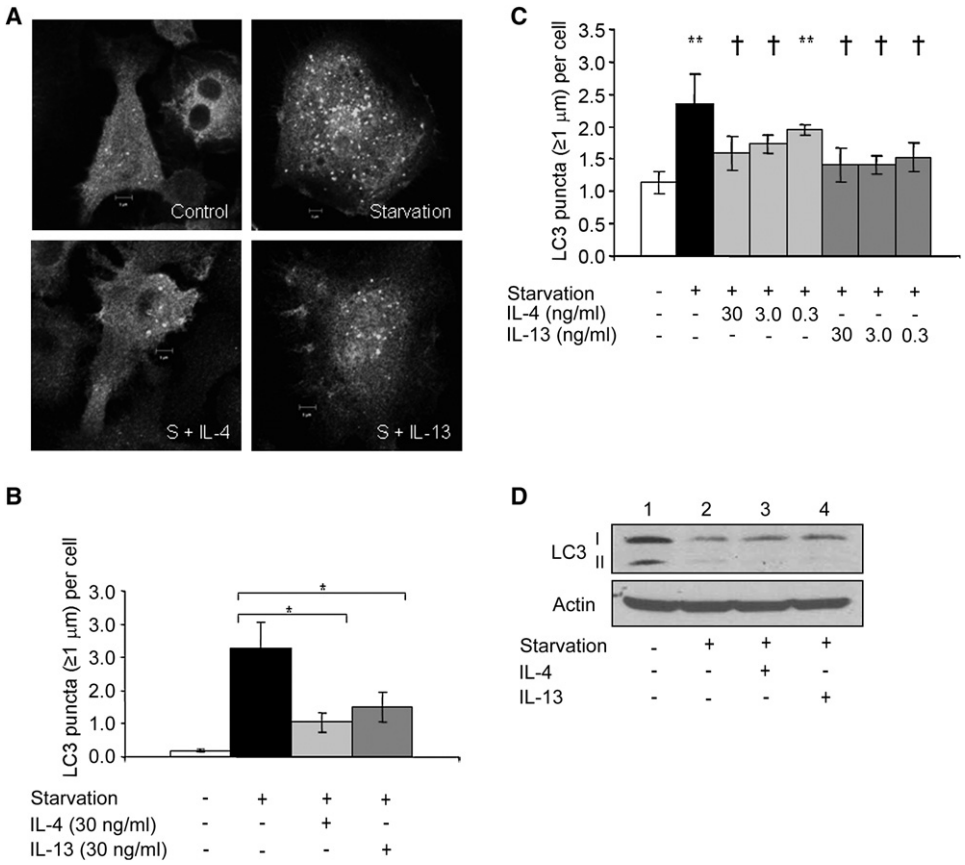

Figure 2. Inhibition of Starvation-Induced Autophagy by IL-4 and IL-13 in Murine Macrophages

(A) Bone marrow macrophages (BMMs) from GFP-LC3 transgenic mice were amino acid- and serum-starved (S) for 2 hr with or without IL-4 or IL-13 (30 ng/ml) and analyzed by confocal microscopy.

(B) Quantification of large (>1 μm) GFP-LC3 puncta per cell (within the denominator; calculations included cells without any large puncta); n = 3 (separate slides).

(C) Quantification of large (≥ 1 μm) LC3 puncta in RAW264.7 cells transfected with GFP-LC3 and induced for autophagy by starvation in the presence of decreasing concentrations of IL-4 or Il-13; n = 3 (separate slides; shown is one out of two independent experiments).

(D) Immunoblot analysis of LC3 lipidation state in RAW264.7 cells starved and incubated without (none) or with IL-4 or IL-13. Full; cells grown in full medium. Cells were amino acid- and serum-starved for 2 hr (lanes 2–4). Actin was used as the loading control. Data are presented as means ± SEM; *$p < 0.05$, **$p < 0.01$, and †$p \geq 0.05$.

(Figure 1). Similar results were obtained with primary bone marrow-derived macrophages (BMMs) from GFP-LC3 transgenic mice (Mizushima et al., 2004) (Figures 2A and 2B). A titration of IL-4 and IL-13 in RAW264.7 cells showed that at 0.3 ng/ml, both cytokines still exerted their effects, albeit IL-4 was losing some of its potency at the lowest concentration tested (Figure 2C).

The inhibitory effects of IL-4 and IL-13 on starvation-induced autophagy were independently confirmed by an assay that measures conversion of LC3-I (nonlipidated form with lower electrophoretic mobility) to LC3-II (LC3 form C-terminally lipidated by phosphatidylethanolamine, displaying higher electrophoretic mobility) with immunoblots (Kabeya et al., 2000). As expected, induction of autophagy by starvation reduced both LC3-I and LC3-II band intensity in RAW264.7 cell (Figure 2D and Figure S2B, lanes 1 and 2) because of the autophagic consumption of LC3 (Kabeya et al., 2000). At the same time, the intensity of the LC3-II band changed (increased) relative to the intensity of LC3-I band (Figure 2D and Figure S2B, lanes 1 and 2). When IL-4 and IL-13 were added to starved macrophages, two changes occurred: (1) The intensity of the LC3-I band increased (Figure 2D, lanes 3 and 4, relative to lane 2), indicative of a reduced LC3-I-to-LC3-II conversion, consistent with the inhibition of autophagy induction by IL-4 and IL-13. An increase in LC3-I band was also observed in primary murine BMM (Figure S2C). (2) The LC3-II band intensity decreased in starved RAW264.7 macrophages treated with IL-4 and IL-13 (Figure 2D, lanes 3 and 4, compared to lane 2). This could be the result of reduced LC3-I-to-LC3-II conversion, in keeping with an inhibition of autophagy induction. However in BMM, only an LC3-I increase but no LC3-II decrease was observed (Figure S2C). The differences in

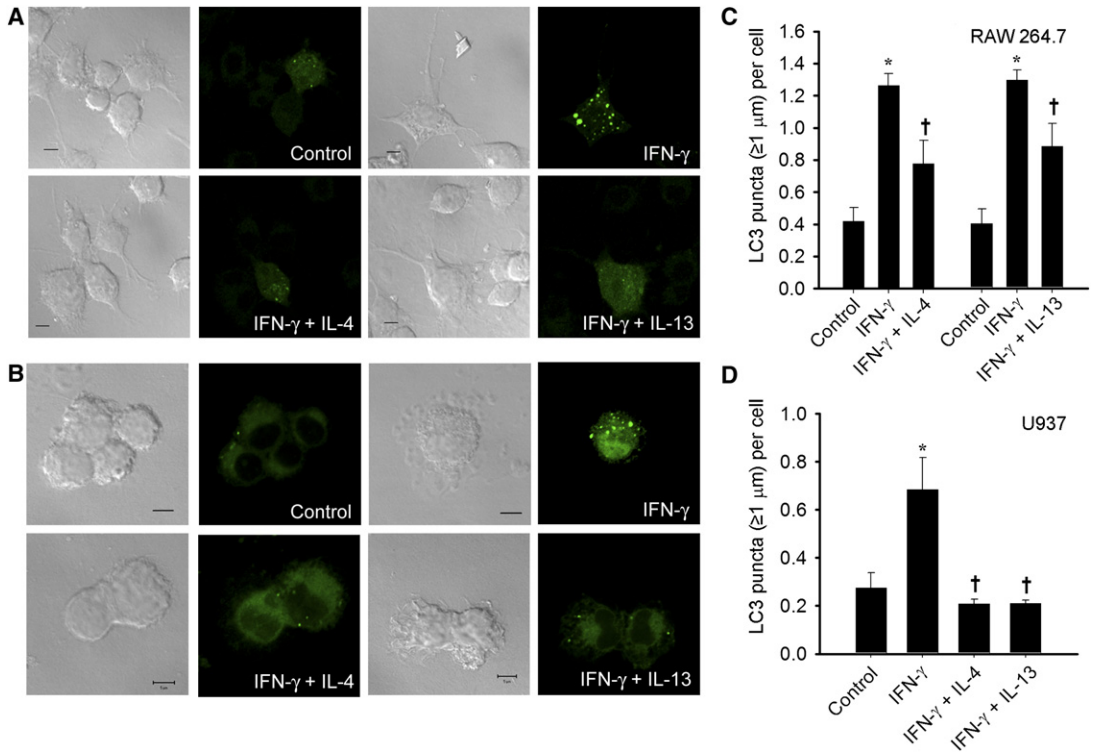

Figure 3. Inhibition of IFN-γ-Induced Autophagy by IL-4 and IL-13

(A) Murine RAW264.7 or (B) human U937 cells were transiently transfected with pEGFP-LC3 and treated with 200 U/ml IFN-γ or IFN-γ in combination with IL-4 or IL-13 (30 ng/ml) for 24 hr and were analyzed by confocal microscopy. Large (≥1 μm) LC3 puncta per cell were quantified (C and D). Data are presented as means ± SEM; *$p < 0.05$, **$p < 0.01$, †$p \geq 0.05$; n = 3. Scale bars represent 5 μm.

LC3-II band intensity between RAW364.7 and BMM may be the net result of differences in the rates of LC3-I-to-LC3-II conversion versus LC3-II depletion through degradation in autolysosomes. This was substantiated with lysosomal and autolysosomal protease inhibitors (Figure S2, lanes 3 and 4, compared to lane 2), which, as expected, increased LC3-II amounts by blocking its degradation both in the presence or absence of IL-4. Consistent with the conclusion that Th2 cytokines inhibit LC3-I-to-LC3-II conversion, IL-4 presence increased LC3-I band intensity (Figure S2B, lane 4 compared to lane 3). Because the presence of IL-4 did not diminish LC3-II band levels relative to protease inhibitors alone (Figure S2, lane 4 versus lane 3), as one might expect from lower LC3-I-to-LC3-II conversion, Th2 cytokines may have an additional effect on the fate of LC3-II by partially inhibiting its delivery to or its degradation in autolysosomes. This conclusion is consistent with the apparent preservation of the LC3-II band in BMM (Figure S2C). Of note is that IL-4 and IL-13 did not change expression of the murine LC3 gene MAP1LC3b under starvation conditions (Figure S2D). In conclusion, IL-4 and IL-13 inhibit LC3-I-to-LC3-II conversion and initiation of autophagy but may have additional effects on the autophagic pathway; these effects become apparent when different cells are examined.

IL-4 and IL-13 Inhibit IFN-γ-Induced Formation of Autophagosomes

Treatment of macrophages with IFN-γ promotes the formation of autophagosomes (Gutierrez et al., 2004; Singh et al., 2006). We tested whether this effect could be inhibited by treating RAW and U937 cells with IFN-γ in combination with either IL-4 or IL-13. In both cell types, IL-4 and IL-13 significantly reduced the number of IFN-γ-induced pEGFP-LC3$^+$ puncta per cell (Figure 3). In addition, we found that IFN-γ treatment increased the percentage of RAW cells with large vacuoles that stained positively for monodansylcadaverine (MDC), another marker for autophagic vacuoles (Biederbick et al., 1995), and this effect was inhibited by IL-4 or IL-13 (Figures S3A and S3B). By using the MDC assay, we confirmed the effects of IL-4 and IL-13 in primary human, peripheral blood monocyte-derived macrophages (MDMs) and found, in titration experiments, a similar concentration-dependence pattern to that seen in murine macrophages (Figure S3C).

IL-4 and IL-13 Inhibit Autophagy-Dependent BCG Phagolysosome Maturation

Mycobacterium tuberculosis normally resides in phagosomes that do not acquire phagolysosomal properties, such as lumenal acidification and the presence of

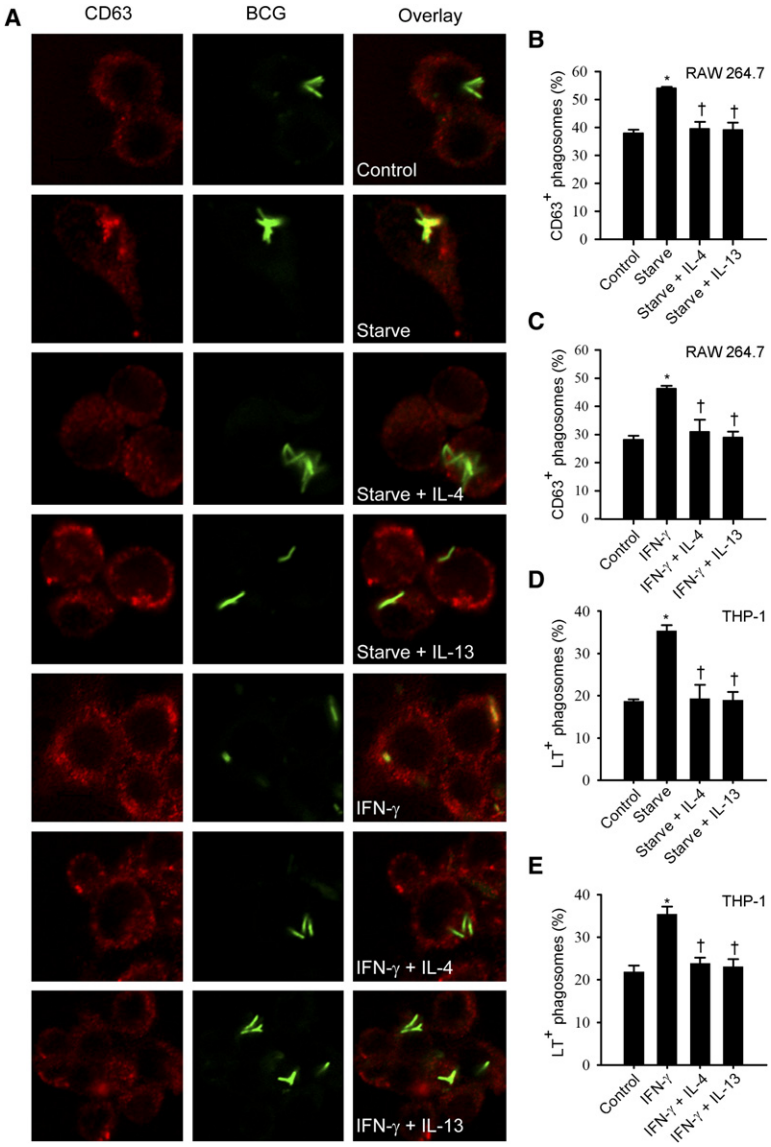

Figure 4. Inhibition of Starvation- and IFN-γ-Induced BCG Phagosome Maturation by IL-4 and IL-13

(A) Confocal images of murine RAW264.7 macrophages infected with GFP-BCG for 2 hr. Cells were amino acid- and serum-starved for 2 hr (during infection) or treated with IFN-γ (200 U/ml) for 24 hr prior to infection, with or without IL-4 or IL-13 (30 ng/ml). After infection, cells were fixed and stained for CD63. The percentage of $CD63^+$ BCG phagosomes was quantified (B and C). The percentage of Lysotracker red $(LT)^+$ BCG phagosomes was recorded in PMA-differentiated human THP-1 monocytes after (D) starvation or (E) IFN-γ (200 U/ml) treatment with or without IL-4 or IL-13 (30 ng/ml). Representative images are shown in Figure S6. Data are presented as means ± SEM; *$p < 0.05$ and †$p \geq 0.05$; $n = 3$.

lysosomal hydrolases (Vergne et al., 2004). Induction of autophagy has been shown to promote the transfer of mycobacteria into degradative autolysosomal organelles (Gutierrez et al., 2004). To investigate whether IL-4 and IL-13 counteract this effect, we examined the maturation of *M. tuberculosis* variant *bovis* BCG (BCG) phagosomes by monitoring the late endosomal or lysosomal marker CD63. Induction of autophagy by starvation in BCG-infected RAW cells significantly increased colocalization of GFP-BCG with CD63 (Figures 4A and 4B). The effect of starvation-induced autophagy on BCG phagosome maturation was inhibited by the addition of either IL-4 or IL-13 (Figures 4A and 4B). Both IL-4 and IL-13 also inhibited IFN-γ-induced phagosome maturation in BCG-infected cells (Figures 4A and 4C). Starvation increased phagosome maturation in U937 cells, an effect that was inhibited with either IL-4 or IL-13 (Figure S4). For unknown reasons, IFN-γ did not significantly increase BCG phagosome maturation in U937 cells (data not shown). Instead, we tested PMA-differentiated human THP-1 cells, by using the acidotropic dye LysoTracker Red (LT) to visualize lysosomal compartments. As with RAW cells, phagosome maturation was increased by either starvation or IFN-γ; this was inhibited by treatment with either IL-4 or IL-13 (Figures 4D and 4E and Figure S5).

Phagosome maturation was autophagy dependent, as demonstrated by siRNA knockdown of Beclin 1, a critical mammalian autophagy factor (Liang et al., 1999) (Figures 5A–5C). Although RAW cells transfected with scrambled siRNA showed an increase in BCG phagosome maturation in response to starvation (Figures 5A and 5D) or IFN-γ (Figures 5B and 5D), cells treated with Beclin 1 siRNA did not, demonstrating that these responses are dependent on functional autophagic machinery. In the

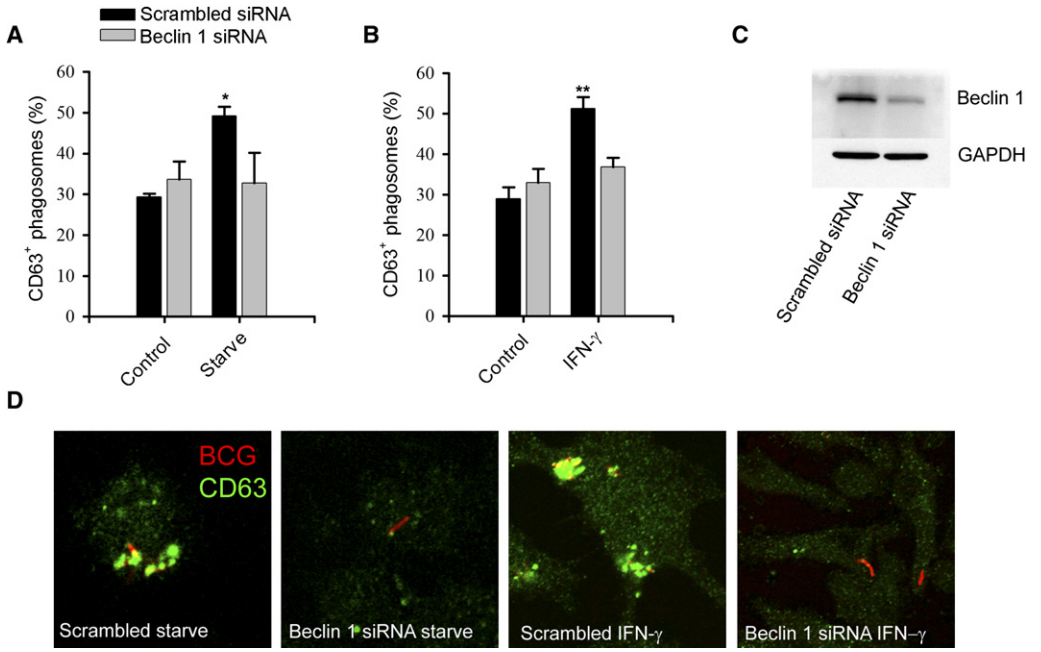

Figure 5. Starvation- and IFN-γ-Induced BCG Phagosome Maturation Is Autophagy Dependent

(A) Quantitative analysis of CD63$^+$ BCG phagosomes that were in murine RAW264.7 macrophages transiently transfected with either nontargeting (scrambled) siRNA or Beclin 1 siRNA and that were amino acid- and serum-starved for 2 hr.

(B) CD63$^+$ BCG phagosomes in RAW264.7 cells transiently transfected with scrambled or Beclin 1 siRNA and treated with IFN-γ (200 U/ml) for 24 hr. Data are presented as means ± SEM; *$p < 0.05$, **$p < 0.01$, and †$p \geq 0.05$; n = 3 for (A) and n = 6 for (B).

(C) Immunoblot confirmation of Beclin 1 knockdown by siRNA.

(D) Immunofluorescence panels of BCG colocalization with the late endosomal marker CD63, exemplifying the data in (A) and (B). CD63 is shown in green; BCG is shown in red.

presence of full-nutrient media, treatment of RAW macrophages with IL-4 or IL-13 had no effect on the colocalization of CD63 with either live or heat-killed BCG phagosomes (Figure S6). Thus, these Th2 cytokines do not inhibit mycobacterial phagosome maturation under normal conditions but rather specifically inhibit autophagy-dependent maturation.

IL-4 and IL-13 Inhibit Autophagy-Dependent Killing of Intracellular Mycobacteria

To determine whether IL-4 and IL-13 affect autophagic elimination of mycobacteria (Gutierrez et al., 2004), we infected RAW cells with BCG and induced autophagy by amino acid starvation for 2 hr. Induction of autophagy decreased BCG survival in RAW cells (Figure 6A). Addition of IL-4 or IL-13 to the starvation media abrogated this effect (Figure 6A). Similar results were obtained when survival of virulent *M. tuberculosis* H37Rv was tested with starvation or IFN-γ used as autophagy agonists (Figures 6B and 6C). Figure 6D shows that these relationships hold true in primary macrophages because IL-4, used as an example, abrogated killing of virulent *M. tuberculosis* H37Rv by starvation-induced autophagy in murine BMMs. These findings demonstrate that IL-4 or IL-13 inhibit autophagy-induced killing of mycobacteria by macrophages.

IL-4 and IL-13 Inhibit Autophagic Phagosome Maturation in Primary Human Cells

To confirm that IL-4 and IL-13 inhibit autophagy-induced effects on mycobacterial phagosomes in human primary macrophages, we tested whether IL-4 and IL-13 influenced autophagy-induced mycobacterial phagosome maturation. Human peripheral blood monocyte-derived macrophages (MDMs) were infected with BCG and induced for autophagy by starvation. The effect of starvation-induced autophagy on mycobacterial phagosome maturation was decreased by the addition of either IL-4 or IL-13 (Figures 6E and 6F). Similarly, both IL-4 and IL-13 had an inhibitory effect on IFN-γ-induced mycobacterial phagosome maturation (Figures 6E and 6F). These results validate in human primary macrophages the conclusion that Th2 cytokines counteract autophagy in its ability to deliver mycobacteria into the phagolysosome.

Inhibition of Starvation-Induced Autophagy by IL-4 and IL-13 Is Akt Dependent

To determine the signaling involved in the actions of IL-4 and IL-13 on autophagy in macrophages, we examined whether the Akt pathway, known to activate TOR and inhibit autophagy (Wullschleger et al., 2006), was involved. In both RAW (Figure 7A) and U937 (Figure S7A) macrophages, IL-4 and IL-13 increased phosphorylation of Akt

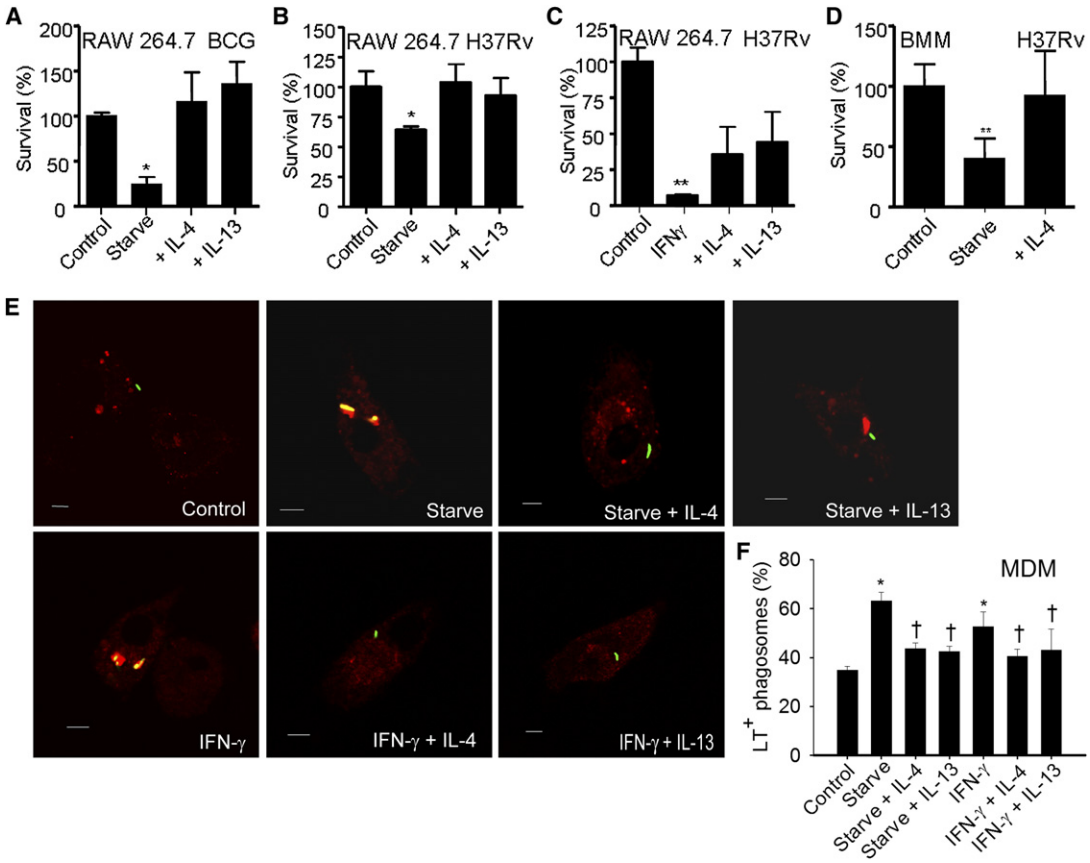

Figure 6. IL-4 and IL-13 Inhibit Autophagy-Dependent Killing of *M. tuberculosis* in Murine Macrophages and Counteract Autophagy-Induced Phagosome Maturation in Primary Human Macrophages

(A) Murine RAW264.7 macrophages were infected with BCG for 1 hr and were amino acid- and serum-starved with or without IL-4 or IL-13 (30 ng/ml) for 2 hr. Cells were washed and lysed for viability determination and survival expressed as a percentage of the control.

(B) Murine RAW264.7 macrophages were infected with virulent *M. tuberculosis* H37Rv for 1 hr and were amino acid- and serum-starved with or without IL-4 or IL-13 (30 ng/ml) for 2 hr. Cells were washed and lysed for viability determination.

(C) Murine RAW264.7 macrophages were either untreated or treated with 200 u/ml of m-IFN-γ for 24 hr prior to infection with *M. tuberculosis* H37Rv for 1 hr with or without IL-4 or IL-13 (30 ng/ml) for 2 hr. Cells were washed and lysed for viability (cfu) determination. Data are presented as means ± SEM, *$p < 0.05$, **$p < 0.01$, and †$p \geq 0.05$; n = 3.

(D) IL-4 inhibits autophagy-dependent killing of *M. tuberculosis* H37Rv in primary murine macrophages. Murine bone marrow macrophages were infected with virulent *M. tuberculosis* (strain H37Rv) for 1 hr and were amino acid- and serum-starved with or without IL-4 (30 ng/ml) for 2 hr. Mycobacterial viability (colony counts) is expressed as a percentage of the control.

(E and F) Primary human macrophages derived from peripheral blood monocytes were infected with BCG. Transfer of mycobacteria to phagolysosome dependent on autophagy was scored with the acidotropic dye LysoTracker. Autophagy was induced for 2 hr by starvation or by treatment with 200 u/ml human IFN-γ. Cells were incubated in the absence or presence of 30 ng/ml of human IL-4 or IL-13, as indicated. (F) shows quantification of experiments shown in (E). Data are presented as means ± SEM; *$p < 0.05$ and †$p \geq 0.05$; n = 3 (three independent donors).

under starvation conditions (Figure S7A, top two panels and graph). We next tested the activity of TOR, a key negative regulator of autophagy (when TOR is active, autophagy is inhibited) on the basis of reports that TOR is activated by Akt (Wullschleger et al., 2006). We investigated TOR activation by monitoring phosphorylation of p70 S6 kinase (S6k) (Figure S7A, bottom two panels and graph) and eukaryotic initiation factor 4E (eIF4E)-binding protein 1 (4E-BP1) (Figures 7B–7E and Figure S7), both commonly used as indicators of TOR activation state (Wullschleger et al., 2006). Treatment of U937 cells with IL-4 or IL-13 increased phosphorylation of S6k (Figure S7A). In RAW264.7 macrophages, IL-4 and IL-13 treatment increased phosphorylation of 4E-BP1 under starvation conditions in a dose-dependent manner (Figures S7B and S7C). A finer-resolution analysis of 4E-BP1 phosphorylation was carried out next. TOR induces 4E-BP1 inactivation via multiple hierarchical phosphorylations (Gingras et al., 2001). A fine-resolution immunoblot analysis can be used for revealing 4E-BP1 hypophosphorylated α and β forms, and hyperphosphorylated γ form, resolved in the order of their decreasing electrophoretic mobility. Active Akt elicits 4E-BP1(γ) hyperphosphorylation through TOR activity (Gingras et al., 2001). Our analyses in primary

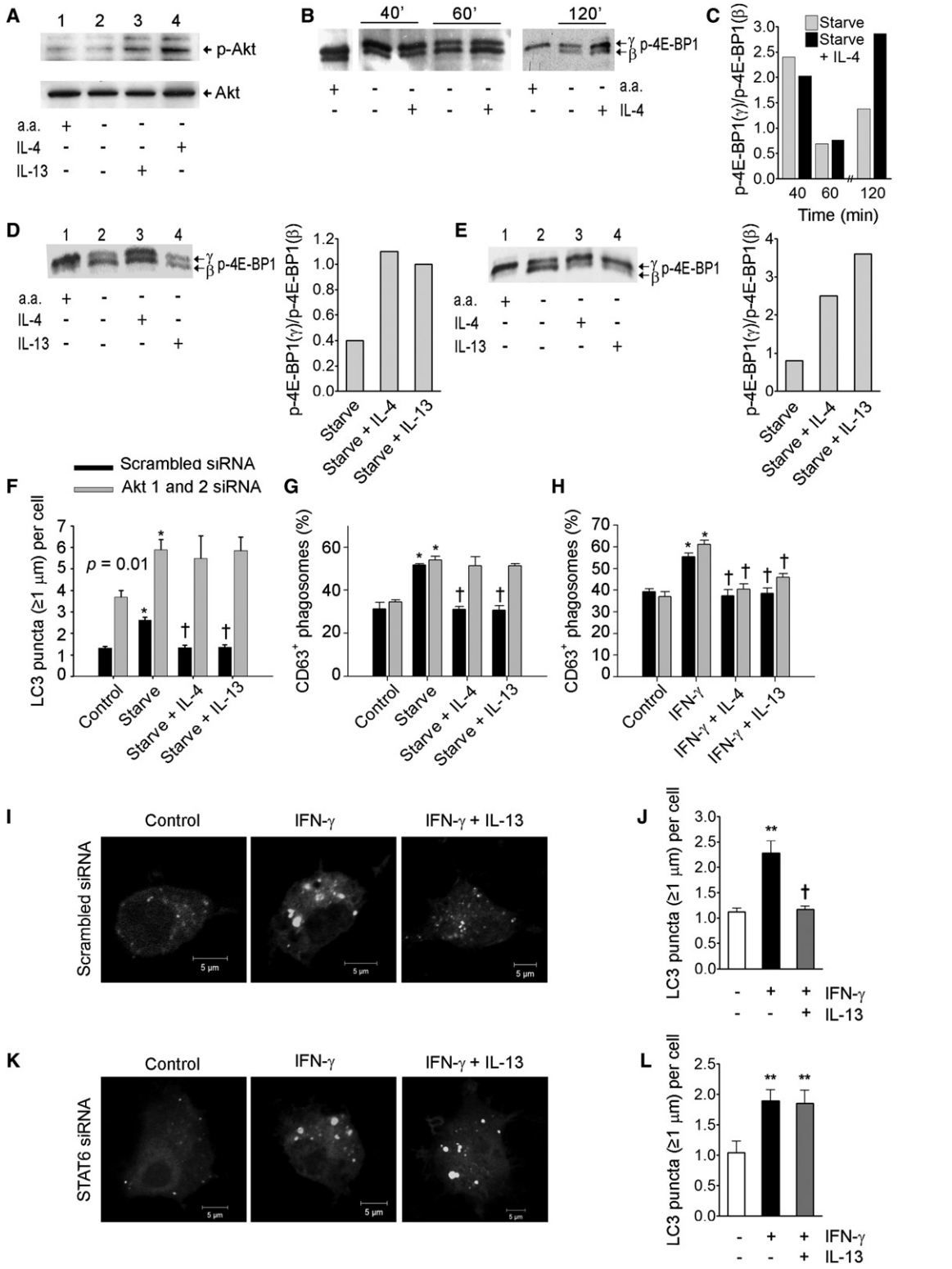

Figure 7. Role of Akt and STAT6 in Suppression by IL-4 and IL-13 of Starvation- and IFN-γ-Induced Autophagy

(A) Immunoblot analysis of Akt phosphorylation in RAW264.7 macrophages amino acid- and serum starved for 2 hr with or without IL-4 or IL-13.

(B) Immunoblot analysis of 4E-BP1 hierarchical phosphorylation in murine bone marrow macrophages (BMMs) starved and treated with IL-4 for 40, 60, and 120 min.

(C) Graph shows the ratio of p-4E-BP1(γ)/p-4E-BP1(β).

(D) Immunoblot analysis of 4E-BP1 in murine bone marrow macrophages (BMMs).

murine BMMs and in human MDM revealed that IL-4 and IL-13 treatment specifically increased phosphorylation of 4E-BP1(γ) under starvation conditions (Figures 7B–7E). Furthermore, a time course of 4E-BP1 showed that IL-4 exerted its effects by increasing 4E-BP1(γ) hyperphosphorylation at 2 hr but had no discernible effects at earlier time points (Figures 7B and 7C), consistent with other effects measured in our study. These results demonstrate that IL-4 and IL-13 activate the Akt pathway in macrophages, resulting in the activation of TOR and inhibition of starvation-induced autophagy.

To establish the role of Akt in the IL-4- and IL-13-induced inhibition of autophagy in macrophages, we knocked down Akt1 and Akt2 with siRNA in vitro (Figure S8A). Compared with the control samples (scrambled siRNA), knockdown of Akt1 and Akt2 in RAW macrophages increased autophagy, monitored by $LC3^+$ vacuole formation (Figure 7F and Figure S8B), indicating that basal levels of autophagy are controlled, at least in part, by baseline Akt activation. Double knockdown of Akt1 and Akt2 in RAW cells abrogated the effects of IL-4 and IL-13 on starvation-induced autophagy (Figure 7F and Figure S8B). In BCG-infected RAW cells, knockdown of Akt1 and Akt2 also inhibited the effects of IL-4 and IL-13 on starvation-induced transfer of BCG to autophagosomes (Figure 7G and Figure S8C).

Inhibition of IFN-γ-Induced Autophagy Is STAT6 Dependent

In contrast to the effects with IL-4 and IL-13 on starvation-induced autophagy, a double knockdown of Akt1 and Akt2 with siRNA had no effect on the inhibition of IFN-γ-induced phagosome maturation by IL-4 and IL-13 (Figure 7H), suggesting that the suppressive effects of these Th2 cytokines on the activation by IFN-γ involved alternative or additional pathways, independent of Akt signaling. Akt3 was detectable only at very low amounts in these cells, and its expression was not affected by knockdown of Akt1 and Akt2 (data not shown). We therefore tested whether the inhibitory effect of IL-4 and IL-13 on IFN-γ-induced autophagy was regulated via a different pathway. IL-4 and IL-13 receptors, in addition to signaling via the Akt pathway, are more commonly known for their activation of STAT6 (Nelms et al., 1999). Thus, we tested whether STAT6 was required for Th2 cytokine inhibition of autophagy activation by IFN-γ. When STAT6 was knocked down (Figure S9), IL-13 could no longer suppress IFN-γ-induced autophagosome formation in macrophages (Figures 7I–7L). Collectively, our data on IL-4 and IL-13 inhibition of starvation- and IFN-γ-induced autophagy demonstrate that Th2 cytokines can employ either of the signaling pathways associated with ligation of the IL-4 and IL-13 receptors to exert this effect. Suppression of starvation-induced autophagy occurs through Akt signaling, whereas STAT6 is needed for suppression of IFN-γ-induced autophagy.

DISCUSSION

Recent studies have demonstrated that autophagy represents a mechanism for eliminating intracellular pathogens (Deretic, 2005; Schmid et al., 2006a; Levine and Deretic, 2007), as shown in several bacterial, viral, and protozoan systems (Birmingham et al., 2006; Gutierrez et al., 2004; Ling et al., 2006; Ogawa et al., 2005; Orvedahl et al., 2007; Paludan et al., 2005; Singh et al., 2006). Induction of autophagy by amino acid starvation, rapamycin treatment, or macrophage activation with IFN-γ leads to an increase in mycobacterial phagolysosome maturation. This increase is concomitant with a decrease in the intracellular survival of the bacilli (Singh et al., 2006), and such a survival decrease is independent of apoptosis (Gutierrez et al., 2004), another process previously implicated in the elimination of *M. tuberculosis* (Fratazzi et al., 1999). Here, we have shown that autophagy-induced killing of mycobacteria by murine and human macrophages is inhibited by the Th2 cytokines IL-4 and IL-13. Both cytokines counteract the autophagic transfer of mycobacteria from their normally immature phagosomes with early endosomal characteristics (Vergne et al., 2004) to autolysosomes, thus preventing the elimination of intracellular bacilli by autophagy.

We have shown that the effect of IL-4 and IL-13 on starvation-induced autophagy is dependent on signaling via the Akt pathway, which activates TOR. Activation of TOR, downstream of Akt, inhibits autophagy. As expected, IL-4 and IL-13 could not influence rapamycin-induced autophagy (data not shown) because rapamycin acts directly on TOR, bypassing Akt signaling. Notably, the mechanism of IL-4 and IL-13 inhibition of IFN-γ-induced autophagy is

(E) Immunnoblot analysis of human monocyte-derived macrophages (MDMs) starved for 2 hr with or without IL-4 or IL-13. Graphs in (D) and (E) show (n = 2; one out of two experiments with similar results) the intensity ratios of p-4E-BP1(γ) to p-4E-BP1(β) bands.
(F) Quantification of large (≥1 μm) pEGFP-LC3 puncta per cell in RAW 264.7 macrophages transiently transfected with pEGFP-LC3 and either scrambled siRNA or Akt 1 + Akt 2 siRNA and starved for 2 hr with or without IL-4 or IL-13.
(G) Quantitative analysis of $CD63^+$ BCG phagosomes in RAW264.7 cells transiently transfected with scrambled siRNA or Akt 1 + Akt 2 siRNA and infected with BCG for 2 hr. Infected cells were amino acid- and serum-starved with or without IL-4 or IL-13.
(H) Percentage of $CD63^+$ BCG phagosomes in RAW264.7 cells transiently transfected with scrambled siRNA or Akt1 + Akt 2 siRNA, treated with IFN-γ with or without IL-4 or IL-13 for 24 hr, and infected with BCG for 2 hr. Representative images from these experiments and immunoblot-blot confirmation of Akt 1 and Akt 2 knockdown are shown in Figure S8.
(I–L) Th2 inhibition of IFNγ-dependent autophagy is STAT6 dependent: (I and J) display confocal-microscopy images and quantification of GFP-LC3 puncta formation in response to IFN-γ, with or without IL-13, in cells treated with control (csramble) siRNA. (K–L) display images and quantification of GFP-LC3 puncta formation in response to IFN-γ, with or without IL-13, in cells treated with control STAT6 siRNA. Immunoblot analysis of STAT6 knockdown is given in Figure S9. Data in all graphs (when error bars are shown) are presented as means ± SEM; $n \geq 3$; *$p < 0.05$, **$p < 0.01$, and †$p \geq 0.05$.

Akt independent. This finding was surprising given the established role of Akt in inhibiting autophagy in general (Petiot et al., 2000) and given the inhibitory effects of Akt in starvation-induced autophagy in macrophages demonstrated here for IL-4 and IL-13 and in nonphagocytic cells elsewhere for IL-13 (Arico et al., 2001). To explain these observations, we have now uncovered a previously unknown signaling relationship; STAT6 modulates the Th2 antiautophagic function when macrophages are stimulated for autophagy by IFN-γ. This places STAT6 within the ranks of the immunologically relevant regulators of autophagy. Most importantly, it provides an explanation for how Th2 cytokines counter autophagy-dependent protection against intracellular pathogens afforded by Th1 responses. The effects of STAT6 on autophagy downstream of IL-4 and IL-13 action may involve a number of pathways, one of which could be linked to the intriguing ability of STAT6 to increase the Bcl-2 and Bcl-X_L antiapoptotic proteins (Wurster et al., 2002). Bcl-2 and Bcl-X_L are now also recognized as being antiautophagic effector proteins (Maiuri et al., 2007; Pattingre et al., 2005). Bcl-2 and Bcl-X_L interact directly (Pattingre et al., 2005) through the recently recognized BH3 domain (Maiuri et al., 2007) within Beclin 1, the key activator of autophagy. Because Bcl-2 and Bcl-X_L association with Beclin 1 blocks autophagy induction (Maiuri et al., 2007), and the rapid induction of STAT6 downstream of IL-4 and IL-13 stimulation may increase Bcl-X_L availability (Wurster et al., 2002), the IL-4-IL-13-STAT6-dependent inhibition of autophagy observed in our work could reflect these recently uncovered relationships.

In this work, we did not specifically study alternative activation of macrophages by IL-4 and IL-13 (Gordon, 2003) but instead focused on the effects of short-term exposure (2 hr) or treatment in combination with IFN-γ. Treatment of macrophages with IL-4 or IL-13 for 6 days has been shown to enhance fluid-phase pinocytosis and mannose receptor-mediated endocytosis through activation of phosphatidylinositol 3 kinase; both cytokines increase tubular-vesicle formation at pericentriolar sites under the plasma membrane, concurrent with decreased particle sorting to the lysosomes (Montaner et al., 1999). Although these previous observations could explain the inhibitory effects on autophagolysosome formation, IL-4 and IL-13 had no effect on BCG phagosome maturation in the absence of starvation- or IFN-γ-induced autophagy, demonstrating that the effects observed in our experiments are autophagy specific. A study on the effects of alternative activation of macrophages with Th2 cytokines, in the context of autophagy, may be difficult to address because prolonged autophagy can lead to type-II-programmed cell death, and most cells will downregulate this process to ensure their survival (Pattingre et al., 2005). Nevertheless, alternative activation of macrophages with IL-4 in vitro delays and inhibits antibacterial responses to intracellular *M. tuberculosis* (Kahnert et al., 2006) by an unknown mechanism that may be related to the processes reported here.

The concentrations of IL-4 and IL-13 used in our titration experiments fall well within the in vivo and ex vivo tissue levels of these cytokines under various conditions (Aspord et al., 2007; Huang et al., 1995; Zhu et al., 1999), including studies with mycobacterial antigens (Chensue et al., 1997). Because local concentrations in the vicinity of T cells secreting IL-4 or IL-13 in vivo can be much higher, our range included 10-fold increments. Although lymphocytes in the lungs of patients with pulmonary tuberculosis typically have a Th1 phenotype, secreting IFN-γ and IL-12 (Mazzarella et al., 2003; Taha et al., 1997), little is known of the phenotype or cytokine-secretion profiles of infected macrophages and dendritic cells within the granuloma. However in vitro studies, in human peripheral blood monocyte-derived macrophages have demonstrated that virulent strains of tuberculosis preferentially upregulate Th2 cytokines (IL-4, IL-5, IL-10, and IL-13), whereas nonvirulent strains induce Th1 cytokines and chemokines (Freeman et al., 2006; Manca et al., 2004; Sun et al., 2006). Moreover, Th2 lymphocyte subsets have been observed in lung tissue from patients with cavitary tuberculosis, compared with Th1 subsets in noncavitary disease (Mazzarella et al., 2003). This correlates with higher production of IL-4 in the periphery of patients with cavitary disease (van Crevel et al., 2000). Thus, it is possible that in an environment predominantly influenced by Th1 cytokines, paracrine, and autocrine secretion of IL-4 and IL-13 could specifically impair the response of infected macrophages to IFN-γ. Moreover, the peripheral response of patients with active tuberculosis disease may not necessarily mirror the response in the lungs. Up to 15% of patients with active pulmonary tuberculosis display specific anergy to tuberculin (Bloom and Small, 1998), and this has been linked with increased ratios of IL-4- and IL-10-positive lymphocytes and decreased ratios of IL-12 (Baliko et al., 1998). Some authors have reported a Th1 response in the periphery of patients with mild pulmonary tuberculosis and Th2 responses in anergic patients and those with more severe disease (Boussiotis et al., 2000; Dlugovitzky et al., 1997). Clinical observations in tuberculin-reactive patients have also demonstrated that Th1 responses, although high in the granuloma, are often paradoxically depressed in peripheral blood lymphocytes in response to mycobacterial antigens (Jo et al., 2003). In this context, it would be interesting to determine whether the cytokine profile in the periphery could influence the autophagic status of newly recruited monocytes in the granuloma. Thus, the balance of Th1 and Th2 cytokines is clearly critical in the host response to *M. tuberculosis*. On the basis of the Th1 autophagy-promoting and Th2 autophagy-dampening roles, we can now shed more light on this dichotomy.

In summary, we have shown that inhibition of autophagy by IL-4 and IL-13 impairs the ability of macrophages to kill intracellular mycobacteria. The Th2 cytokines inhibit physiologically and immunologically induced autophagy in murine and human monocyte and macrophage cells. Moreover, these cytokines inhibit autophagy-dependent maturation of the mycobacterial phagosome and subsequent killing of mycobacteria. In addition to furthering our understanding of the modulation of autophagy in macrophages, these data present evidence for a novel role of

Th1-Th2 polarization, modulating autophagy as an immune effector mechanism in opposing ways. Our studies offer an explanation as to why Th2 cytokines are incompatible with protection against certain intracellular pathogens, including *M. tuberculosis.* By antagonizing autophagy, IL-4 and IL-13 inhibit Th1-dependent protection against mycobacteria. Because Th1 and Th2 cytokines show antagonistic effects on autophagy as an immune effector, the regulation of autophagy may represent a critical aspect of Th1-Th2 polarization in the host response to intracellular pathogens.

EXPERIMENTAL PROCEDURES

Antibodies and Reagents

Unless otherwise stated, reagents were from Sigma. Recombinant murine and human IL-4 and IL-13 were purchased from R & D Systems. Rabbit polyclonal antibody against CD63 was from Santa Cruz Biotechnology, rabbit polyclonal antibody against LC3 was from T. Ueno and E. Kominami. pEGFP-LC3 was from T. Yoshimori and GFP-LC3 transgenic mice were from N. Mizushima. Tdtomato-LC3 was from G. Bjorkoy.

Cells and Bacterial Cultures

Murine RAW264.7 macrophages were cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) supplemented with 10% fetal bovine serum (FBS) and L-glutamine (full-nutrient medium). The human monoblastic cell lines, U937 and THP-1, were maintained in RPMI-1640 (Invitrogen) with 10% FBS, L-glutamine, and HEPES. Before use, U937 and THP-1 cells were differentiated with PMA (100 nM) for 24–72 hr. *Mycobacterium bovis* BCG was grown in Middlebrook 7H9 broth with 0.5% Tween, 0.2% glycerol, and albumin-dextrose-catalase supplement (BD Diagnostics, Franklin Lakes, NJ, USA). Primary human monocyte-derived macrophages (MDMs) were isolated and cultured from buffy coats by density gradient centrifugation on Ficoll-Paque Plus (GE Healthcare). PBMCs were allowed to adhere to gelatin-coated plates for at least 1 hr, washed so that nonadherent cells could be removed, and cultured in RPMI-1640 with 10% human human AB serum for 24 hr. After this time, cells were lifted, counted, and plated on tissue-culture plastic or glass coverslips for 5–7 days. Bone marrow-derived macrophages (BMMs) were isolated and cultured as described.

Flow Cytometry

After blocking with seroblock (AbD Serotec), RAW264.7 cells were stained with rat anti-mouse CD124 (IL-4Ra)-PE (BD) for 30 min on ice. U937 and THP-1 cells were blocked with human serum (10%) and stained first with anti-human CD124 for 30 min on ice, then with anti-mouse IgG1-PE (BD) for 30 min on ice. The cells were analyzed on a BD FACScaliber, and data were processed with CellQuest software.

Induction of Autophagy

Autophagy was induced either by amino acid starvation, in which cells were incubated for 2 hr in Earle's balanced salt solution (EBSS) or by treatment with IFN-γ (200 U/ml) for 24 hr. (Gutierrez et al., 2004).

Macrophage Transfection

RAW264.7 and U937 cells were transfected by nucleoporation as previously described (Chua and Deretic, 2004). In brief, cells were harvested after 2–3 days in culture and resuspended in 100 μl of the appropriate electroporation buffer (Amaxa Biosystems) with 5–10 μg plasmid DNA or 1.5 μg siGENOME SMARTpool siRNA or siCONTROL nontargeting siRNA (Dharmacon) and nucleofected with Amaxa Nucleofector apparatus. After electroporation, cells were cultured in full-nutrient medium for 24 hr before use.

Fluorescence Confocal Microscopy

Cells were cultured on glass coverslips, fixed in 2% paraformaldehyde for 20 min at room temperature, permeabilized, and blocked in PBS with 0.5% Tween 20, 1% BSA, and 2% goat serum for 30 min at room temperature. Cells were incubated with primary antibody for 1 hr and then with secondary antibody for an additional 1 hr. Alternatively, cells were incubated with Lysotracker Red DND-99 (LT, Invitrogen) for 2 hr prior to and during incubation with mycobacteria. For labeling of acidic, lipid-rich vacuoles, cells were incubated with monodansylcadaverine (MDC, 50 μM) for 15 min prior to fixing. Bone marrow macrophages expressing GFP-LC3 were fixed with 2% paraformaldehyde for 10 min, permeabilized with 0.2% saponin for 5 min, blocked for 30 min, and incubated with rabbit polyclonal antibodies against GFP (Abcam) overnight for visualization of the GFP-LC3 fusion protein (GFP fluorescence in BMMs isolated from these transgenic mice is sporadic, and GFP antibody is routinely used for visualization of GFP-LC3.). Slides were incubated with a secondary anti-mouse fluorescein isothiocyanate-conjugated antibody. Coverslips were mounted onto glass slides with Permafluor Aqueous mounting medium (Thermo Scientific, Waltham, MA, USA) and analyzed on a Zeiss LSM510 META laser-scanning confocal microscope.

Immunoblotting

Cells were washed in PBS and lysed with buffer containing 10 mM Tris HCl, 150 mM NaCl, 0.5% deoxycholate, 2 mM EDTA, 2% NP-40, 1 mM PMSF, and protease inhibitor cocktail (Roche Applied Science). A total of 50 μg of protein was loaded and separated on a 12% or 15% SDS-polyacrylamide gel (BioRad) and transferred to nitrocellulose. The membrane was blocked in 5% milk or 3% BSA in PBS/Tween 20 (0.1%) and probed with antibodies overnight at 4°C. After washing with PBS/Tween, the blot was probed with HRP-conjugated secondary antibody for 1 hr at room temperature. Staining was revealed with SuperSignal West Dura Extended Duration Substrate (Pierce).

Phagolysosome Maturation and Mycobacterial-Survival assays

For phagocytosis of mycobacteria, macrophages were incubated with Texas red-labeled BCG, GFP-BCG, or *Mycobacterium tuberculosis* strain H37Rv for 15–30 min, washed, and incubated for an additional 2 hr, in the presence of starvation media in the presence or absence of cytokines as indicated. After staining the fixed cells for the lysosomal marker CD63 (LAMP3), confocal microscopy was used so that the percentage of CD63-positive mycobacterial phagosomes from at least 100 cells could be recorded. For mycobacterial-survival assays, RAW cells or MDM were infected with BCG or H37Rv for 1 hr, washed, and incubated for an additional 2 hr with starvation media and cytokines as indicated. Cells were washed with PBS and lysed with distilled water. Serial dilutions of lysates were made, and 5 ml aliquots were inoculated on Middlebrook 7H10 agar plates supplemented with oleic acid–albumin–dextrose–catalase (BD). Plates were sealed and incubated for 2 weeks at 37°C, and colonies were counted from dilutions yielding 10–50 visible colonies. Data are expressed as colony-forming units per ml (cfu/ml).

Statistical Analysis

Data are presented as means ± SEM (approximately three independent experiments); p values (Student's t test; two-tailed) are relative to the control, unless otherwise specified.

Supplemental Data

Nine figures and one movie are available at http://www.immunity.com/cgi/content/full/27/3/505/DC1/.

ACKNOWLEDGMENTS

We thank G. Bjorkoy for tdTomato-LC3, T. Ueno for LC3 antibody, T. Yoshimori for GFP-LC3, N. Mizushima for GFP-LC3 transgenic mice, and M. Mudd, A. Davis, and G. Kyei for preparation of primary

macrophages. This work was supported by grant AI069345 and in part by grants AI045148 and AI042999 from the National Institutes of Health (NIH). S. De Haro was supported by AI045148S1 supplement award from NIH. E.A. Roberts was a Heiser Foundation Postdoctoral Fellow in Tuberculosis and Leprosy Research.

Received: January 10, 2007
Revised: June 20, 2007
Accepted: July 25, 2007
Published online: September 20, 2007

REFERENCES

Andrade, R.M., Wessendarp, M., Gubbels, M.J., Striepen, B., and Subauste, C.S. (2006). CD40 induces macrophage anti-Toxoplasma gondii activity by triggering autophagy-dependent fusion of pathogen-containing vacuoles and lysosomes. J. Clin. Invest. *116*, 2366–2377.

Arico, S., Petiot, A., Bauvy, C., Dubbelhuis, P.F., Meijer, A.J., Codogno, P., and Ogier-Denis, E. (2001). The tumor suppressor PTEN positively regulates macroautophagy by inhibiting the phosphatidylinositol 3-kinase/protein kinase B pathway. J. Biol. Chem. *276*, 35243–35246.

Aspord, C., Pedroza-Gonzalez, A., Gallegos, M., Tindle, S., Burton, E.C., Su, D., Marches, F., Banchereau, J., and Palucka, A.K. (2007). Breast cancer instructs dendritic cells to prime interleukin 13-secreting CD4+ T cells that facilitate tumor development. J. Exp. Med. *204*, 1037–1047.

Baliko, Z., Szereday, L., and Szekeres-Bartho, J. (1998). Th2 biased immune response in cases with active Mycobacterium tuberculosis infection and tuberculin anergy. FEMS Immunol. Med. Microbiol. *22*, 199–204.

Biederbick, A., Kern, H.F., and Elsasser, H.P. (1995). Monodansylcadaverine (MDC) is a specific in vivo marker for autophagic vacuoles. Eur. J. Cell Biol. *66*, 3–14.

Birmingham, C.L., Smith, A.C., Bakowski, M.A., Yoshimori, T., and Brumell, J.H. (2006). Autophagy controls salmonella infection in response to damage to the salmonella-containing vacuole. J. Biol. Chem. *281*, 11374–11383.

Bjorkoy, G., Lamark, T., Brech, A., Outzen, H., Perander, M., Overvatn, A., Stenmark, H., and Johansen, T. (2005). p62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death. J. Cell Biol. *171*, 603–614.

Blommaart, E.F., Krause, U., Schellens, J.P., Vreeling-Sindelarova, H., and Meijer, A.J. (1997). The phosphatidylinositol 3-kinase inhibitors wortmannin and LY294002 inhibit autophagy in isolated rat hepatocytes. Eur. J. Biochem. *243*, 240–246.

Bloom, B.R., and Small, P.M. (1998). The evolving relation between humans and Mycobacterium tuberculosis. N. Engl. J. Med. *338*, 677–678.

Boussiotis, V.A., Tsai, E.Y., Yunis, E.J., Thim, S., Delgado, J.C., Dascher, C.C., Berezovskaya, A., Rousset, D., Reynes, J.M., and Goldfeld, A.E. (2000). IL-10-producing T cells suppress immune responses in anergic tuberculosis patients. J. Clin. Invest. *105*, 1317–1325.

Chensue, S.W., Warmington, K., Ruth, J.H., Lukacs, N., and Kunkel, S.L. (1997). Mycobacterial and schistosomal antigen-elicited granuloma formation in IFN-gamma and IL-4 knockout mice: Analysis of local and regional cytokine and chemokine networks. J. Immunol. *159*, 3565–3573.

Chua, J., and Deretic, V. (2004). Mycobacterium tuberculosis reprograms waves of phosphatidylinositol 3-phosphate on phagosomal organelles. J. Biol. Chem. *279*, 36982–36992.

Dengjel, J., Schoor, O., Fischer, R., Reich, M., Kraus, M., Muller, M., Kreymborg, K., Altenberend, F., Brandenburg, J., Kalbacher, H., et al. (2005). Autophagy promotes MHC class II presentation of peptides from intracellular source proteins. Proc. Natl. Acad. Sci. USA *102*, 7922–7927.

Deretic, V. (2005). Autophagy in innate and adaptive immunity. Trends Immunol. *26*, 523–528.

Djavaheri-Mergny, M., Amelotti, M., Mathieu, J., Besancon, F., Bauvy, C., Souquere, S., Pierron, G., and Codogno, P. (2006). NF-kappaB activation represses tumor necrosis factor-alpha-induced autophagy. J. Biol. Chem. *281*, 30373–30382.

Dlugovitzky, D., Torres-Morales, A., Rateni, L., Farroni, M.A., Largacha, C., Molteni, O., and Bottasso, O. (1997). Circulating profile of Th1 and Th2 cytokines in tuberculosis patients with different degrees of pulmonary involvement. FEMS Immunol. Med. Microbiol. *18*, 203–207.

Donaldson, D.D., Whitters, M.J., Fitz, L.J., Neben, T.Y., Finnerty, H., Henderson, S.L., O'Hara, R.M., Jr., Beier, D.R., Turner, K.J., Wood, C.R., and Collins, M. (1998). The murine IL-13 receptor alpha 2: Molecular cloning, characterization, and comparison with murine IL-13 receptor alpha 1. J. Immunol. *161*, 2317–2324.

Fichtner-Feigl, S., Strober, W., Kawakami, K., Puri, R.K., and Kitani, A. (2006). IL-13 signaling through the IL-13alpha2 receptor is involved in induction of TGF-beta1 production and fibrosis. Nat. Med. *12*, 99–106.

Fratazzi, C., Arbeit, R.D., Carini, C., Balcewicz-Sablinska, M.K., Keane, J., Kornfeld, H., and Remold, H.G. (1999). Macrophage apoptosis in mycobacterial infections. J. Leukoc. Biol. *66*, 763–764.

Freeman, S., Post, F.A., Bekker, L.G., Harbacheuski, R., Steyn, L.M., Ryffel, B., Connell, N.D., Kreiswirth, B.N., and Kaplan, G. (2006). Mycobacterium tuberculosis H37Ra and H37Rv differential growth and cytokine/chemokine induction in murine macrophages in vitro. J. Interferon Cytokine Res. *26*, 27–33.

Gingras, A.C., Raught, B., Gygi, S.P., Niedzwiecka, A., Miron, M., Burley, S.K., Polakiewicz, R.D., Wyslouch-Cieszynska, A., Aebersold, R., and Sonenberg, N. (2001). Hierarchical phosphorylation of the translation inhibitor 4E–BP1. Genes Dev. *15*, 2852–2864.

Gordon, S. (2003). Alternative activation of macrophages. Nat. Rev. Immunol. *3*, 23–35.

Gutierrez, M.G., Master, S.S., Singh, S.B., Taylor, G.A., Colombo, M.I., and Deretic, V. (2004). Autophagy is a defense mechanism inhibiting BCG and Mycobacterium tuberculosis survival in infected macrophages. Cell *119*, 753–766.

Huang, S.K., Xiao, H.Q., Kleine-Tebbe, J., Paciotti, G., Marsh, D.G., Lichtenstein, L.M., and Liu, M.C. (1995). IL-13 expression at the sites of allergen challenge in patients with asthma. J. Immunol. *155*, 2688–2694.

Inbal, B., Bialik, S., Sabanay, I., Shani, G., and Kimchi, A. (2002). DAP kinase and DRP-1 mediate membrane blebbing and the formation of autophagic vesicles during programmed cell death. J. Cell Biol. *157*, 455–468.

Jo, E.K., Park, J.K., and Dockrell, H.M. (2003). Dynamics of cytokine generation in patients with active pulmonary tuberculosis. Curr. Opin. Infect. Dis. *16*, 205–210.

Kabeya, Y., Mizushima, N., Ueno, T., Yamamoto, A., Kirisako, T., Noda, T., Kominami, E., Ohsumi, Y., and Yoshimori, T. (2000). LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. EMBO J. *19*, 5720–5728.

Kahnert, A., Seiler, P., Stein, M., Bandermann, S., Hahnke, K., Mollenkopf, H., and Kaufmann, S.H. (2006). Alternative activation deprives macrophages of a coordinated defense program to Mycobacterium tuberculosis. Eur. J. Immunol. *36*, 631–647.

Kawakami, K., Taguchi, J., Murata, T., and Puri, R.K. (2001). The interleukin-13 receptor alpha2 chain: An essential component for binding and internalization but not for interleukin-13-induced signal transduction through the STAT6 pathway. Blood *97*, 2673–2679.

Kuma, A., Hatano, M., Matsui, M., Yamamoto, A., Nakaya, H., Yoshimori, T., Ohsumi, Y., Tokuhisa, T., and Mizushima, N. (2004). The role of autophagy during the early neonatal starvation period. Nature *432*, 1032–1036.

Kyei, G.B., Vergne, I., Chua, J., Roberts, E., Harris, J., Junutula, J.R., and Deretic, V. (2006). Rab14 is critical for maintenance of Mycobacterium tuberculosis phagosome maturation arrest. EMBO J. *25*, 5250–5259.

Levine, B. (2005). Eating oneself and uninvited guests: Autophagy-related pathways in cellular defense. Cell *120*, 159–162.

Levine, B., and Deretic, V. (2007). Unveiling the roles of autophagy in innate and adaptive immunity. Nat. Rev. Immunol., in press. Published online September 3, 2007. 10.1038/nri2161.

Liang, X.H., Jackson, S., Seaman, M., Brown, K., Kempkes, B., Hibshoosh, H., and Levine, B. (1999). Induction of autophagy and inhibition of tumorigenesis by beclin 1. Nature *402*, 672–676.

Ling, Y.M., Shaw, M.H., Ayala, C., Coppens, I., Taylor, G.A., Ferguson, D.J., and Yap, G.S. (2006). Vacuolar and plasma membrane stripping and autophagic elimination of Toxoplasma gondii in primed effector macrophages. J. Exp. Med. *203*, 2063–2071.

Lum, J.J., Bauer, D.E., Kong, M., Harris, M.H., Li, C., Lindsten, T., and Thompson, C.B. (2005). Growth factor regulation of autophagy and cell survival in the absence of apoptosis. Cell *120*, 237–248.

Maiuri, M.C., Le Toumelin, G., Criollo, A., Rain, J.C., Gautier, F., Juin, P., Tasdemir, E., Pierron, G., Troulinaki, K., Tavernarakis, N., et al. (2007). Functional and physical interaction between Bcl-X(L) and a BH3-like domain in Beclin-1. EMBO J. *26*, 2527–2539.

Manca, C., Reed, M.B., Freeman, S., Mathema, B., Kreiswirth, B., Barry, C.E., 3rd, and Kaplan, G. (2004). Differential monocyte activation underlies strain-specific Mycobacterium tuberculosis pathogenesis. Infect. Immun. *72*, 5511–5514.

Mazzarella, G., Bianco, A., Perna, F., D'Auria, D., Grella, E., Moscariello, E., and Sanduzzi, A. (2003). T lymphocyte phenotypic profile in lung segments affected by cavitary and non-cavitary tuberculosis. Clin. Exp. Immunol. *132*, 283–288.

Mizushima, N., Yamamoto, A., Matsui, M., Yoshimori, T., and Ohsumi, Y. (2004). In vivo analysis of autophagy in response to nutrient starvation using transgenic mice expressing a fluorescent autophagosome marker. Mol. Biol. Cell *15*, 1101–1111.

Montaner, L.J., da Silva, R.P., Sun, J., Sutterwala, S., Hollinshead, M., Vaux, D., and Gordon, S. (1999). Type 1 and type 2 cytokine regulation of macrophage endocytosis: Differential activation by IL-4/IL-13 as opposed to IFN-gamma or IL-10. J. Immunol. *162*, 4606–4613.

Nelms, K., Keegan, A.D., Zamorano, J., Ryan, J.J., and Paul, W.E. (1999). The IL-4 receptor: Signaling mechanisms and biologic functions. Annu. Rev. Immunol. *17*, 701–738.

Ogawa, M., Yoshimori, T., Suzuki, T., Sagara, H., Mizushima, N., and Sasakawa, C. (2005). Escape of intracellular Shigella from autophagy. Science *307*, 727–731.

Orvedahl, A., Alexander, D., Tallóczy, Z., Sun, Q., Wei, Y., Zhang, W., Burns, D., Leib, D., and Levine, B. (2007). HSV-1 ICP34.5 confers neurovirulence by targeting the Beclin 1 autophagy protein. Cell Host Microbe *1*, 23–35.

Paludan, C., Schmid, D., Landthaler, M., Vockerodt, M., Kube, D., Tuschl, T., and Munz, C. (2005). Endogenous MHC class II processing of a viral nuclear antigen after autophagy. Science *307*, 593–596.

Pattingre, S., Tassa, A., Qu, X., Garuti, R., Liang, X.H., Mizushima, N., Packer, M., Schneider, M.D., and Levine, B. (2005). Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. Cell *122*, 927–939.

Petiot, A., Ogier-Denis, E., Blommaart, E.F., Meijer, A.J., and Codogno, P. (2000). Distinct classes of phosphatidylinositol 3′-kinases are involved in signaling pathways that control macroautophagy in HT-29 cells. J. Biol. Chem. *275*, 992–998.

Pyo, J.O., Jang, M.H., Kwon, Y.K., Lee, H.J., Jun, J.I., Woo, H.N., Cho, D.H., Choi, B., Lee, H., Kim, J.H., et al. (2005). Essential roles of Atg5 and FADD in autophagic cell death: Dissection of autophagic cell death into vacuole formation and cell death. J. Biol. Chem. *280*, 20722–20729.

Roberts, E.A., Chua, J., Kyei, G.B., and Deretic, V. (2006). Higher order Rab programming in phagolysosome biogenesis. J. Cell Biol. *174*, 923–929.

Schmid, D., Dengjel, J., Schoor, O., Stevanovic, S., and Munz, C. (2006a). Autophagy in innate and adaptive immunity against intracellular pathogens. J. Mol. Med. *84*, 194–202.

Schmid, D., Pypaert, M., and Munz, C. (2006b). Antigen-loading compartments for major histocompatibility complex class II molecules Continuously receive input from autophagosomes. Immunity *26*, 79–92.

Shintani, T., and Klionsky, D.J. (2004). Autophagy in health and disease: A double-edged sword. Science *306*, 990–995.

Singh, S.B., Davis, A.S., Taylor, G.A., and Deretic, V. (2006). Human IRGM induces autophagy to eliminate intracellular mycobacteria. Science *313*, 1438–1441.

Sun, Y.J., Lim, T.K., Ong, A.K., Ho, B.C., Seah, G.T., and Paton, N.I. (2006). Tuberculosis associated with Mycobacterium tuberculosis Beijing and non-Beijing genotypes: A clinical and immunological comparison. BMC Infect. Dis. *6*, 105.

Taha, R.A., Kotsimbos, T.C., Song, Y.L., Menzies, D., and Hamid, Q. (1997). IFN-gamma and IL-12 are increased in active compared with inactive tuberculosis. Am. J. Respir. Crit. Care Med. *155*, 1135–1139.

van Crevel, R., Karyadi, E., Preyers, F., Leenders, M., Kullberg, B.J., Nelwan, R.H., and van der Meer, J.W. (2000). Increased production of interleukin 4 by CD4+ and CD8+ T cells from patients with tuberculosis is related to the presence of pulmonary cavities. J. Infect. Dis. *181*, 1194–1197.

Vergne, I., Chua, J., Singh, S.B., and Deretic, V. (2004). Cell biology of mycobacterium tuberculosis phagosome. Annu. Rev. Cell Dev. Biol. *20*, 367–394.

Wullschleger, S., Loewith, R., and Hall, M.N. (2006). TOR signaling in growth and metabolism. Cell *124*, 471–484.

Wurster, A.L., Rodgers, V.L., White, M.F., Rothstein, T.L., and Grusby, M.J. (2002). Interleukin-4-mediated protection of primary B cells from apoptosis through Stat6-dependent up-regulation of Bcl-xL. J. Biol. Chem. *277*, 27169–27175.

Zhang, J.G., Hilton, D.J., Willson, T.A., McFarlane, C., Roberts, B.A., Moritz, R.L., Simpson, R.J., Alexander, W.S., Metcalf, D., and Nicola, N.A. (1997). Identification, purification, and characterization of a soluble interleukin (IL)-13-binding protein. Evidence that it is distinct from the cloned Il-13 receptor and Il-4 receptor alpha-chains. J. Biol. Chem. *272*, 9474–9480.

Zhu, Z., Homer, R.J., Wang, Z., Chen, Q., Geba, G.P., Wang, J., Zhang, Y., and Elias, J.A. (1999). Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. J. Clin. Invest. *103*, 779–788.

Note Added in Proof

The following errors as they appeared in the original publication of this paper (on September 20, 2007) have now been corrected online: (1) Figure S2 did not contain panel D; (2) Figures S5, S6, and S7 were not in order and were incorrectly cited in the main text (detailed below in points 3–6); (3) Figure S5 as it was published originally online should have been Figure S7; (4) Figure S6 should have been Figure S5; (5) Figure S7 should have been Figure S6; (6) on page 511 of the original main text, all citations for Figure S7 were incorrectly cited as Figure S5; (7) and in Figure 3 of the main text, panels A and B were incorrectly labeled-all instances of the label "Starve" should have read "IFN-γ." See the Correction in the October 26th issue for further details.