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The Lung Is Protected from Spontaneous Inflammation by Autophagy in Myeloid Cells

Masashi Kanayama,* You-Wen He,* and Mari L. Shinohara*†

The lung is constantly exposed to environmental oxygen, dust, and microbes. Therefore, to avoid inflammatory responses to harmless and ambient levels of stimulation, the lung developed site-specific immune regulatory strategies to restrain inflammation, imparted by unique resident cellular populations. Alveolar macrophages (AMs) are lung-resident macrophages. In terms of inflammation in the lung, AMs have two opposing functions (1). They inhibit inflammation in the lung and are equipped with inhibitory factors to terminate ongoing inflammation by upregulating anti-inflammatory receptors, such as CD200R, TREM2, and MARCO, on the cell surface. AMs are also known as an inducer of regulatory T cells (Tregs) by supplying TGF-β and retinoic acid (2). In contrast, AMs are activated by pattern recognition receptor (PRR)-mediated signaling and produce proinflammatory cytokines and chemokines as immune sentinels in the lung (1). The phagocytic function of AMs also contributes to clear viral, bacterial, and fungal pathogens (3). Proinflammatory immune responses by AMs result in the recruitment of other immune cell types, such as neutrophils and inflammatory monocytes; therefore, AMs have to fine-tune the threshold above which an infection is perceived as a threat in detecting ligands of PRRs and in exerting immune responses.

Autophagy is a cellular process that degrades unwanted cytoplasmic components, such as old proteins, organelles, and intracellular pathogens. Autophagy is induced by signaling through PRRs and cytokine receptors to eliminate intracellular microbes through autophagosomal digestion (4–11). Recent studies showed that autophagy regulates immune responses in pathological conditions. For example, autophagy removes reactive oxygen species (ROS)-generating mitochondria and suppresses inflammasome-mediated IL-1β/IL-18 production (12) to downregulate proinflammatory responses. In viral and bacterial infections, autophagy mediates Ag processing and presentation to enhance adoptive immune responses (13, 14). In fungal infection, we recently reported that autophagy enhances NF-κB–mediated chemokine production in tissue-resident macrophages by sequestering an NF-κB inhibitor, A20 (15). In particular, autophagy in pulmonary myeloid cells, including AMs, is known to prevent excessive immune responses and inflammation under pathological conditions, such as endotoxemia, cystic fibrosis, and hemorrhagic shock (16–19). However, the impact of autophagy on the maintenance of immune homeostasis under nonpathological condition remains unclear.

In this study, we showed that mice lacking Atg7 in myeloid cells spontaneously develop pulmonary inflammation. Without ATG7, lung inflammation was initiated between 2 and 3 wk of age and was largely mediated by infiltration of innate immune cells, such as neutrophils, Ly6C+ monocytes, and dendritic cells (DCs). Interestingly, the Atg7 conditional knockout (CKO) mice did not induce inflammation in organs other than the lung. ATG7 in myeloid cells plays a role in maintaining a state of immune ignorance or tolerance to harmless stimuli in the lung by lowering bacterial/fungal loads in the lung, decreasing mitochondrial ROS (mtROS) production, and increasing the detection threshold of PRR ligands perceived as a threat. Indeed, administration of antibiotics and treatment with a ROS inhibitor prevented the initiation of pulmonary inflammation in Atg7 CKO mice. Thus, autophagy inhibits spontaneous inflammation in the lung through controlling the loads of environmental microbes and the sensitivity of AMs, even in non-pathological conditions.

Materials and Methods

Animals and reagents

All the mice were on the C57BL/6 background. Atg7fl/fl mice were described previously (20). Ly5Mcr mice were purchased from The Jackson Laboratory. Experiments were performed as approved by the Institutional Animal Care and Use Committee. Abs against CD45, CD11b, F4/80, TLR4, TLR2,
CD4, CD3, CD11c, Siglec F, CD200R, Ly6G, and Ly6C were purchased from BioLegend. Dectin-1 and dectin-2 Abs were from AbD Serotec. Abs against TREM2 and MARCO were purchased from R&D Systems. MitoSOX and dihydroethidium (DHE) were purchased from Life Technologies. A ROS inhibitor, N-acetyl-l-cysteine (NAC), was purchased from Sigma.

Cell culture condition

AMs were sorted by FACS with a MoFlo Legacy (Beckman Coulter) as CD45+CD11c+CD200-Fc+ and cultured in RPMI 1640 medium containing 10% FCS, penicillin/streptomycin (Sigma), and 2 mM L-glutamine. For some experiments, AMs were stimulated with or without LPS (0–10 ng/ml) containing 10% FCS, penicillin/streptomycin (Sigma), and 2 mM L-glutamine. For ligation of LPS, 15 min of LPS (1 ng/ml) was administered to a mouse from weeks 2 to 3 after birth. For intranasal instillation, LPS, 15 μl LPS (1 ng/ml) was administered daily to a mouse from days 7 to 12 after birth.

Flow cytometry analysis

Lung tissues were minced and treated with collagenase D (1 mg/ml) for 30 min at 37°C. Cells were enriched by density gradient centrifugation with Percoll (GE Healthcare), and enumerated with a hemocytometer by Trypan Blue exclusion. After staining with specific Abs, cells were analyzed by FACSCanto II (BD) with FlowJo software (TreeStar).

Evaluation of ROS production

Lungs were obtained from 7-mo-old wild-type (WT) and Atg7 CKO mice for more detail, we examined gene expression and found that various proinflammatory cytokines (Tnfa, Il6, Spp1) (denoted as WT hereafter) and neutrophils (polymorphonuclear cells [PMNs]) (Supplemental Fig. 1A, 1B). Atg7 CKO mice appeared healthy and had normal reproductive ability. Nevertheless, we noticed that Atg7 CKO mice showed significantly higher gene expression of proinflammatory molecules 7 wk after birth only in the lungs (Fig. 1A). To assess the differences between Atg7 CKO mice and LysM+CKO mice (denoted as WT hereafter) for more detail, we examined gene expression and found that various proinflammatory cytokines (Tnfa, Il6, Spp1 [Opn]) and chemokines (Cxcl1, Cxcl2, Ccl2) started decreasing in the lungs of Atg7 CKO mice at 3 wk after birth (Fig. 1B).

To further evaluate inflammation, we examined cell infiltration in the lungs. Significantly increased numbers of total cells started to be identified in the lungs of 7-wk-old Atg7 CKO mice (Fig. 2A) consistently in every experiment. In particular, more neutrophils (Ly6G+CD11b+) were identified in the lungs of 3-wk-old Atg7 CKO mice (Fig. 2B), reflecting the induction of Cxcl1 and Cxcl2 (Fig. 1B), neutrophil chemoattractants. By 7 wk of age, the numbers of Ly6C+ macrophages (Ly6C+Ly6G-CD11b+), AMs (CD45+CD11c+Ly6F4/80-SiglecF+), and conventional DCs (cDCs; CD45+Ly6G-CD11c+Ly6F/80+) were significantly higher in the lungs of Atg7 CKO mice compared with WT mice (Fig. 2B) (gating strategy is shown in Supplemental Fig. 1C–E). In contrast, the numbers of lymphocytes, such as CD4+ and CD8+ T cells, B cells, and NK cells, were

Statistical analysis

The two-tailed Student t test was used for statistical analyses.

Results

Atg7-deficient mice spontaneously develop pulmonary inflammation

We generated Atg7+/homozygous M (LysM)+CKO mice (denoted as Atg7 CKO mice hereafter) and confirmed that Atg7 mRNA expression and autophagy induction were greatly attenuated in various myeloid cells, such as AMs, bone marrow–derived macrophages (BMMs), BM-derived DCs (BMDCs), and neutrophils (polymorphonuclear cells [PMNs]) (Supplemental Fig. 1A, 1B). Atg7 CKO mice appeared healthy and had normal reproductive ability. Nevertheless, we noticed that Atg7 CKO mice showed significantly higher gene expression of proinflammatory molecules 7 wk after birth only in the lungs (Fig. 1A). To assess the differences between Atg7 CKO mice and LysM+CKO mice (denoted as WT hereafter) for more detail, we examined gene expression and found that various proinflammatory cytokines (Tnfa, Il6, Spp1 [Opn]) and chemokines (Cxcl1, Cxcl2, Ccl2) started decreasing in the lungs of Atg7 CKO mice at 3 wk after birth (Fig. 1B).

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Intranasal treatment with antibiotics, NAC, and LPS

A mixture of sulfamethoxazole (0.8 mg/ml) and trimethoprim (0.16 mg/ml) was used for in vivo antibiotics treatment. For intranasal instillation of antibiotics or NAC, 15 μl antibiotics or NAC solution (50 mM) was administered to a mouse from weeks 2 to 3 after birth. For intranasal instillation of LPS, 15 μl LPS (1 ng/ml) was administered daily to a mouse from days 7 to 12 after birth.
comparable in the lungs of 7-wk-old WT and Atg7 CKO mice (Supplemental Fig. 1F, 1G). These results suggested that the pulmonary inflammation in Atg7 CKO mice is largely mediated by innate immune cells. In accordance with a previous study reporting GM-CSF–dependent proliferation of AMs (24), the expression of Csf2 mRNA, which encodes GM-CSF, was elevated in the lungs of Atg7 CKO mice from 3 wk after birth (Fig. 2C). Corresponding to the lung-specific upregulation of inflammatory gene expression in Atg7 CKO mice (Fig. 1A), we also confirmed the lung-specific increase in the infiltration of innate immune cells (Fig. 2D). However, Atg7 CKO mice showed normal histology in airway surface areas, even in aged mice (7 mo) (Supplemental Fig. 2A, 2B), suggesting that the spontaneous inflammation in Atg7 CKO mice was not severe enough to result in visible tissue damage. Taken together, the lack of Atg7 induces spontaneous pulmonary inflammation that is characterized by the activated expression of proinflammatory genes and myeloid cell infiltration into the lungs.

**Littermate comparisons between Atg7 CKO and WT mice**

The cage-specific environment was ruled out to explain the spontaneous inflammation in Atg7 CKO mice. To compare offspring born from the same parents and raised in the same cage, we bred Atg7fl/flLysMcre/+ born from the same parents and raised in the same cage, we bred Atg7fl/flLysMcre/+ mice with WT strains to obtain C57BL/6 mice under specific pathogen–free conditions (5–8). First, we confirmed that LPS induces autophagy in AMs from 3-wk-old WT mice by identifying LC3 puncta formation (Supplemental Fig. 1H). It is possible that clearance of environmental pathogens in the lungs failed due to the absence of autophagy in Atg7 CKO mice; thus, we evaluated total bacterial burdens in the lungs by quantitative PCR (qPCR). The burdens were higher in the lungs of Atg7 CKO mice than in WT mice 3 wk after birth (Fig. 5A), although increases in bacterial burdens were not observed at other time points (2 and 7 wk after birth) or in other organs (spleen, kidney, and mesenteric lymph nodes) (Supplemental Fig. 2C). We assessed the burdens of Proteobacteria and Firmicutes, major components of lung-resident bacteria, in C57BL/6 mice under specific pathogen–free condition and TREM2, are upregulated on the surface of AMs to control proinflammatory responses (25–27). Expression levels of these receptors were actually higher than WT mice (Fig. 4A), suggesting that Atg7-deficient AMs are well equipped to induce the expression of inhibitory receptors. AMs also suppress inflammation by inducing the generation of Tregs (2) and Tfh, respectively, compared with WT AMs (Fig. 4E). Thus, mechanisms that negatively regulate immune responses appeared to be functioning normally in Atg7-deficient AMs.

**Negative regulation of inflammatory responses is normal in Atg7-deficient AMs**

AMs can negatively control pulmonary inflammation. In inflammatory conditions, inhibitory receptors, such as CD200R, MARCO, and TREM2, are upregulated on the surface of AMs to control proinflammatory responses (25–27). Expression levels of these receptors were actually higher than WT mice (Fig. 4A), suggesting that Atg7-deficient AMs are well equipped to induce the expression of inhibitory receptors. AMs also suppress inflammation by inducing the generation of Tregs (2); thus, we next examined the levels of Tregs in the lungs of 7-wk-old Atg7 CKO mice. The percentages and absolute numbers of Tregs were increased significantly in Atg7 CKO mice compared with WT mice (Fig. 4B, 4C), again indicating no defect in Treg numbers in the lungs of Atg7 CKO mice. In addition, mRNA expression of Il10 in the whole lung tissue was elevated in 7-wk-old Atg7 CKO mice (Fig. 4D). AMs purified from Atg7 CKO mice immediately prior to the onset of pulmonary inflammation at 2 wk of age showed comparable and even higher expression of Il10 and Tgfβ, respectively, compared with WT AMs (Fig. 4E). Thus, mechanisms that negatively regulate immune responses appeared to be functioning normally in Atg7-deficient AMs.

**Contribution of environmental microbes to the development of spontaneous pulmonary inflammation**

Autophagy is known to sequester various microbial pathogens by containing microbes in autophagosomes (4, 9–11). Autophagy plays a critical role in clearing pathogens, particularly in pulmonary myeloid cells, including AMs (5–8). First, we confirmed that LPS induces autophagy in AMs from 3-wk-old WT mice by identifying LC3 puncta formation (Supplemental Fig. 1H). It is possible that clearance of environmental pathogens in the lungs failed due to the absence of autophagy in Atg7 CKO mice; thus, we evaluated total bacterial burdens in the lungs by quantitative PCR (qPCR). The burdens were higher in the lungs of Atg7 CKO mice than in WT mice 3 wk after birth (Fig. 5A), although increases in bacterial burdens were not observed at other time points (2 and 7 wk after birth) or in other organs (spleen, kidney, and mesenteric lymph nodes) (Supplemental Fig. 2C). We assessed the burdens of Proteobacteria and Firmicutes, major components of lung-resident bacteria, in C57BL/6 mice under specific pathogen–free condition...
(28) by qPCR using specific primers. The burdens of both bacterial phyla were increased in the lungs of 3 wk-old Atg7 CKO mice compared with age-matched WT mice (Fig. 5B). Importantly, the increase in lung bacterial burdens in Atg7 CKO mice coincides with the apparent pulmonary inflammation in mice at ∼3 wk after birth (Figs. 1B, 2A–C); thus, it is possible that increased bacterial burdens triggered spontaneous inflammation in the lungs of Atg7 CKO mice. To evaluate the involvement of bacteria in the development of spontaneous lung inflammation, WT and Atg7 CKO mice were intranasally instilled with antibiotics every day from 2 to 3 wk after birth. The treatment successfully reduced bacterial burdens intranasally instilled with antibiotics every day between 2 and 3 wk after birth. The treatment success fully reduced bacterial burdens (Fig. 5C), gene expression of Il10 and Cxcl1, and lung neutrophil counts in Atg7 CKO mice to levels similar to those in WT mice (Fig. 5D, 5E). Similarly, antibiotics added to drinking water from a prenatal stage to 3 wk after birth reduced the expression of pro-inflammatory cytokines in Atg7 CKO mice nearly to the levels seen in WT mice (Supplemental Fig. 2D–F). These results suggested that spontaneous pulmonary inflammation in Atg7 CKO mice is triggered, at least in part, by environmental bacteria and that the inflammation could be prevented by antibiotics.

Activated phenotype of AMs in Atg7 CKO mice

Although cell surface expression of CD11b on AMs from WT mice was not observed, AMs from Atg7 CKO mice expressed significantly high levels of CD11b (Supplemental Fig. 3A, 3B). Induction of CD11b expression in AMs from Atg7 CKO mice became apparent at 3 wk after birth (Supplemental Fig. 3C) and may reflect stimulation of AMs by elevated GM-CSF in the lungs (Fig. 2C), as previously reported (29). AMs from 7-wk-old Atg7 CKO mice also showed increased expression levels of CD11c, F4/80 (Supplemental Fig. 3D), increased granularity, and increased cell sizes (Supplemental Fig. 3E), suggesting that Atg7 CKO AMs were exposed to stimulation. Interestingly, the increase in granularity was observed even in 2-wk-old mice, which had yet to exhibit detectable lung inflammation. Thus, it is possible that AMs start to be stimulated as early as 2 wk after birth in Atg7 CKO mice, and spontaneous pulmonary inflammation manifests at 3 wk after birth. Interestingly, lung-resident DCs in Atg7 CKO mice showed no difference in CD11c expression, granularity, or cell size compared with WT mice (Supplemental Fig. 3F, 3G). These results suggested that, compared with lung-resident DCs, AMs are vulnerable and require autophagy to protect them from spontaneous activation.

Autophagy in AMs plays a role in tolerance of low-intensity environmental stimuli

So far, our data suggested that Atg7-deficient AMs overrespond to stimulation at environmental levels. Therefore, we examined the sensitivity of Atg7 CKO mice to low levels of LPS. Seven-day-old WT or Atg7 CKO mice were instilled intranasally with low-dose LPS (15 pg/mouse) daily for 5 d, and gene expression was evaluated when the mice were 12 d old (Fig. 6A), at which time pulmonary inflammation does not occur if naive (Figs. 1B, 2A, 2B). With LPS treatment, Atg7 CKO mice exhibited increased expression of Tnfα and Il6 mRNA in the lungs, whereas WT mice did not (Fig. 6A). Because the bacterial burdens in the lungs of WT and Atg7 CKO mice are comparable at 2 wk of age (Fig. 5A), these results suggested that the lack of autophagy lowered the detection threshold of LPS in the lungs. To identify the involvement of AMs in the increased sensitivity of Atg7 CKO lungs, AMs from 2-wk-old WT

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**FIGURE 4.** Anti-inflammatory responses in the lungs of Atg7 CKO mice. (A) Expression levels of CD200R, MARCO, and TREM2 on the surface of AMs obtained from 7-wk-old Atg7 CKO and WT mice. (B) Representative flow cytometry results for Foxp3+ Tregs in the lungs of 7-wk-old Atg7 CKO and WT mice. (C) Frequency and total numbers of Foxp3+ Tregs in the lungs of 7-wk-old Atg7 CKO and WT mice. (D) Il10 mRNA expression in lung tissues obtained from Atg7 CKO (n = 4) and WT (n = 3) mice. (E) Levels of Il10 and Tgfb mRNA in AMs obtained from 2- or 3-wk-old Atg7 CKO and WT mice. Each sample was pooled from three mice. Bars in (A)–(C) represent mean ± SD. Bars in (D) and (E) denote RQ-Max/Min, as described in Methods. Data are representative of two independent experiments. *p < 0.05.

**FIGURE 5.** Atg7 deficiency increases bacterial burdens in the lung. (A) and (B) Bacterial burdens in the lungs of WT and Atg7 CKO mice. Burdens of total bacteria (A) and Proteobacteria and Firmicutes (B) were evaluated by qPCR using universal bacterial 16S rRNA primers and phylum-specific 16S rRNA primers, respectively (normalized to mouse Actb). (C) Bacterial burdens in antibiotics-treated WT and Atg7 CKO mice. Mice were instilled intranasally with antibiotics daily from 2 to 3 wk after birth. mRNA was isolated from the whole lung, and bacterial burdens were evaluated by qPCR. (D) Expression levels of Il6 and Cxcl1 in the lungs from WT and Atg7 CKO mice, with or without antibiotic treatment. Data are shown as gene expression in Atg7 CKO mice relative to WT mice. (E) Numbers of neutrophils (PMNs) in the lungs of WT and Atg7 CKO mice with or without antibiotic treatment (n = 3/group). Data are representative of at least two independent experiments and are mean ± SD. *p < 0.05.
Atg7 CKO mice were stimulated with 10 ng/ml of LPS ex vivo. Indeed, Atg7-deficient AMs produced higher levels of TNF-\(\alpha\) and CXCL2 than did WT AMs, although there were no differences in basal expression levels of the cytokines (Fig. 6B). Next, we evaluated the LPS sensitivity of Atg7-deficient AMs using titrated and low concentrations of LPS ex vivo. Atg7-deficient AMs were extremely sensitive to low concentrations (0.3 and 1 ng/ml) of LPS, as shown by the production of TNF-\(\alpha\), whereas WT AMs were much less sensitive (Fig. 6C). Reflecting the sensitivity of Atg7 CKO AMs, the gene expression of proinflammatory cytokines and chemokines was elevated in Atg7-deficient AMs from 2- and 3-wk-old mice (Fig. 6D), although the expression levels of PRRs in Atg7-deficient AMs were comparable to those in WT AMs (Supplemental Fig. 3H). Importantly, in contrast to AMs, BM-derived Atg7-deficient macrophages and DCs did not show increased LPS sensitivity, as reflected by their TNF-\(\alpha\) levels (Supplemental Fig. 4A, 4B). Peritoneal-resident macrophages from 2-wk-old Atg7 CKO mice also did not show increased sensitivity in the absence of Atg7 (Supplemental Fig. 4C). These results indicated that the impact of autophagy in LPS sensitivity varies among cell types.

Autophagy appears to play a role in the tolerance of low levels of stimulants in AMs.

Prevention of spontaneous pulmonary inflammation by inhibiting ROS

Autophagy inhibits mtROS production by degrading damaged mitochondria (12). Indeed, Atg7-deficient AMs showed increased levels of total and mitochondrial ROS at 2 and 7 wk of age compared with WT AMs (Fig. 6E, 6F). Although we do not rule out NADPH oxidase as another source of total ROS, the data suggest that mitochondria are a major source of ROS. Indeed, autophagy is known to sequester damaging mitochondria (30). Interestingly, splenic and peritoneal tissue-resident macrophages obtained from 2-wk-old WT and Atg7 CKO mice were treated intranasally with 15 \(\mu\)M of 50 mM NAC daily for 7 days, starting at 2 wk after birth. Lungs were harvested at the completion of NAC treatment. (J) Levels of the indicated mRNAs as expression ratios between Atg7-deficient AMs and WT AMs (n = 3–4/group). (K) Numbers of neutrophils in the lung (n = 3–4/group). Data are representative of at least two independent experiments and are mean \(\pm\) SD. *\(p\) < 0.05.
treatment, the gene expression of Il6, Tnfa, Cxcl1, and Cxcl2 was much higher in Atg7-deficient AMs than in WT AMs, but NAC treatment successfully narrowed the difference between Atg7-deficient AMs and WT AMs (Fig. 6f). Importantly, NAC treatment reduced neutrophil recruitment in the lungs of Atg7 CKO mice but not WT mice (Fig. 6k). Because a recent study showed that NAC antagonizes proteasome activity (31) and enhances inflammatory responses (32), the inhibition of proteasome by NAC in the pulmonary inflammation of Atg7 CKO mice may also be involved. Collectively, these results suggest that autophagy also downregulates mtROS in AMs to further contribute to the inhibition of spontaneous inflammation in an AM-specific manner.

Discussion

Autophagy regulates innate immune responses. For example, autophagy inhibits inflammasome activation by degrading ROS-producing mitochondria and inflammasomes under septic conditions (12, 33). In contrast, autophagy also boosts NF-kB–mediated innate immune responses in tissue-resident macrophages and contributes to the host protection against Candida infection, as we reported recently (15). The role of autophagy in immune responses has been studied intensively in pathological conditions. However, it has been largely unknown whether and how autophagy is involved in the maintenance of immune homeostasis in non-pathological conditions. In this study, we found that Atg7 deficiency in myeloid cells induces the development of spontaneous pulmonary inflammation as the result of increased bacterial burdens, enhanced ROS production, and increased sensitivity of AMs during the preweaning period. Although we do not rule out the involvement of LC3-associated phagocytosis, which also requires ATG7 (34), these results suggest that autophagy in premature pups is part of the lung-specific mechanism to maintain immune ignorance or tolerance not to overrespond to harmless environmental stimuli.

Based on our data, 7-wk-old adult WT and Atg7 CKO mice reduced bacteria to an undetectable level in the lungs (Fig. 5a). The clearance in WT mice may reflect the maturation of the immune system; however, in Atg7 CKO mice, in particular, clearance may be achieved by the increase in innate immune cells, such as neutrophils and monocytes, recruited to the lung (Fig. 2b). However, 3-wk-old Atg7 CKO mice still had significantly high bacterial burdens (Fig. 5a), and this is the time when lung inflammation becomes apparent. Antibiotics prevented spontaneous lung inflammation in Atg7 CKO mice (Fig. 5c–e, Supplemental Fig. 2d–f), suggesting the critical involvement of the environmental levels of bacteria in young pups. In addition to the increased bacterial burdens, AMs from Atg7 CKO mice are sensitive to low-level LPS stimulation (Fig. 6b, 6c). Our data suggested that these two factors predispose the lungs of preweaned Atg7 CKO pups to spontaneous inflammation later in life.

It is of note that our results manifest a clear contrast between the lung and other organs, as well as between AMs and other macrophage subsets. Atg7 CKO mice showed inflammation only in the lungs. (Figs. 1a, 2d). Young Atg7 CKO mice had higher bacterial burdens in the lungs compared with WT mice, but not in other organs (Supplemental Fig. 2c). In addition, Atg7 deficiency increased mtROS production and sensitivity to TLR4 ligand in AMs (Fig. 6b–f), but not in other myeloid cells, such as BMDMs, BMDCs, peritoneal-resident macrophages, and splenic macrophages (Supplemental Fig. 4a–d). These findings suggest that the lung is a special organ that takes advantage of autophagy to maintain its homeostasis, and AMs appear to be critical. This also reflects the unique setting of the lung, with frequent exposure to microbes and dusts in the air, and possibly the distinct biology of AMs that evolved to meet the specific needs of the lung.

In conclusion, Atg7 deficiency in myeloid cells causes mice to spontaneously develop pulmonary inflammation. Autophagy appeared to protect mice from spontaneous lung inflammation, at least in part, through the following three mechanisms: maintenance of low burdens of environmental microbes in the lung, preventing overresponse of AMs to TLR4 stimulation, and keeping mtROS production low in AMs to control inflammation. These mechanisms may mutually influence one another to prevent inflammatory responses. Furthermore, we found that spontaneous pulmonary inflammation in Atg7 CKO mice can be prevented by treatment with antibiotics or NAC.

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Disclosures

The authors have no financial conflicts of interest.

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