

Autophagy-Mediated Activation of Mucosal-Associated Invariant T Cells Driven by Mesenchymal Stem Cell-Derived IL-15

Guiwen Ye,^{2,4} Peng Wang,^{1,4} Zhongyu Xie,^{1,4} Qian Cao,³ Jinteng Li,¹ Guan Zheng,¹ Shan Wang,³ Ming Li,² Wenjie Liu,¹ Shuizhong Cen,² Zhaofeng Li,² Wenhui Yu,¹ Yanfeng Wu,^{3,*} and Huiyong Shen^{1,2,*}

¹Department of Orthopedics, The Eighth Affiliated Hospital, Sun Yat-sen University, Shenzhen 518033, P.R. China

²Department of Orthopedics, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou 510120, P.R. China

³Center for Biotherapy, The Eighth Affiliated Hospital, Sun Yat-sen University, Shenzhen 518033, P.R. China

⁴These authors contributed equally

*Correspondence: wuyf@mail.sysu.edu.cn (Y.W.), shenhuiy@mail.sysu.edu.cn (H.S.)

<https://doi.org/10.1016/j.stemcr.2021.03.005>

SUMMARY

Mucosal-associated invariant T (MAIT) cells are innate-like unconventional T cells that are abundant in humans and have attracted increasing attention in recent years. Mesenchymal stem cells (MSCs) are crucial regulators of immune cells. However, whether MAIT cells are regulated by MSCs is unclear. Here, we explored the effect of MSCs on MAIT cells and revealed the underlying mechanism. We found that MSCs did not influence the proliferation of MAIT cells but strikingly induced an activated phenotype with an increased expression of CD69, TNF- α , IFN- γ , and granzyme B. Moreover, MSCs activated MAIT cells in a TCR-MR1-independent mechanism through MSC-secreted IL-15. We revealed that MSC-derived IL-15 activated MAIT cells by enhancing autophagy activity, which was abolished by the autophagy inhibitor 3-methyladenine. Based on our findings, MAIT cells are activated by MSCs through IL-15-induced autophagy, which may help elucidate the mechanisms underlying some immune responses and diseases and provide guidance for future research.

INTRODUCTION

Mucosal-associated invariant T (MAIT) cells represent a unique subtype of T cells that express a semi-invariant T cell receptor (TCR) with a V α 7.2 chain in humans and are restricted to the major histocompatibility complex class 1-like molecule MR1 (Franciszkiewicz et al., 2016; Toubal et al., 2019). Critically, MAIT cells are abundant in humans, and their frequencies among T cells are 1%–10% in peripheral blood, 20%–45% in the liver, and 3%–5% in the intestine (Debusschere et al., 2016). In addition, MAIT cells play an important role in antimicrobial infection (Meermeier et al., 2018) and participate in many metabolic disorders and inflammatory diseases, such as diabetes mellitus and rheumatoid arthritis (RA) (Chiba et al., 2018).

By expressing a special TCR chain, MAIT cells can be activated in a TCR-dependent manner via MR1 presenting vitamin B2 metabolites (Franciszkiewicz et al., 2016). In addition, MAIT cells can be activated in a TCR-independent manner through cytokines, including interleukin-7 (IL-7), IL-12, IL-15, and IL-18 (Gracey et al., 2016; Le Bourhis et al., 2011; Li et al., 2019b). After activation, MAIT cells produce high levels of inflammatory cytokines, including tumor necrosis factor alpha (TNF- α), interferon- γ (IFN- γ), IL-17, and the cytolytic factor granzyme B (GB) (Gracey et al., 2016). T cells are always regulated by immunoregulatory cells, which is important for their functions in organisms (Wang et al., 2012). However, knowledge of the role of MAIT cells in this process is limited.

Mesenchymal stem cells (MSCs) represent a type of stem cells derived from various tissues, such as umbilical cord blood and bone marrow (Pittenger et al., 2019). Specifically, MSCs exert strong regulatory effects on various types of immune cells and are regarded as vital regulators of the immune response (Song et al., 2020). Previously, numerous studies have shown that MSCs modulate the survival, differentiation, and function of T lymphocytes, B lymphocytes, macrophages, etc. (Glenn and Whartenby, 2014). Recently, researchers have remained focused on studying the interaction between MSCs and the immune system and obtained several meaningful results. Carreras-Planella et al. (2019) showed that MSCs induced regulatory B cells via soluble factors other than extracellular vesicles. Kiernan et al. (2020) reported that MSCs alter helper T cell subsets in CD4+ memory T cells and Shahir et al. (2020) showed that MSC-derived exosomes could modulate the induction of tolerogenic dendritic cells. Extensive studies of the interaction between MSCs and immune cells are meaningful and necessary. However, researchers have not investigated whether MAIT cells are regulated by MSCs.

In this study, we explored the effect of MSCs on MAIT cells and found that MSCs activate MAIT cells. Mechanistically, MSC-derived IL-15 enhanced the autophagy level of MAIT cells and resulted in MAIT cell activation. Our findings reveal the regulatory effect of MSCs on MAIT cells for the first time and uncover the underlying mechanism, which may help elucidate some immune responses and diseases and provide guidance for future research.



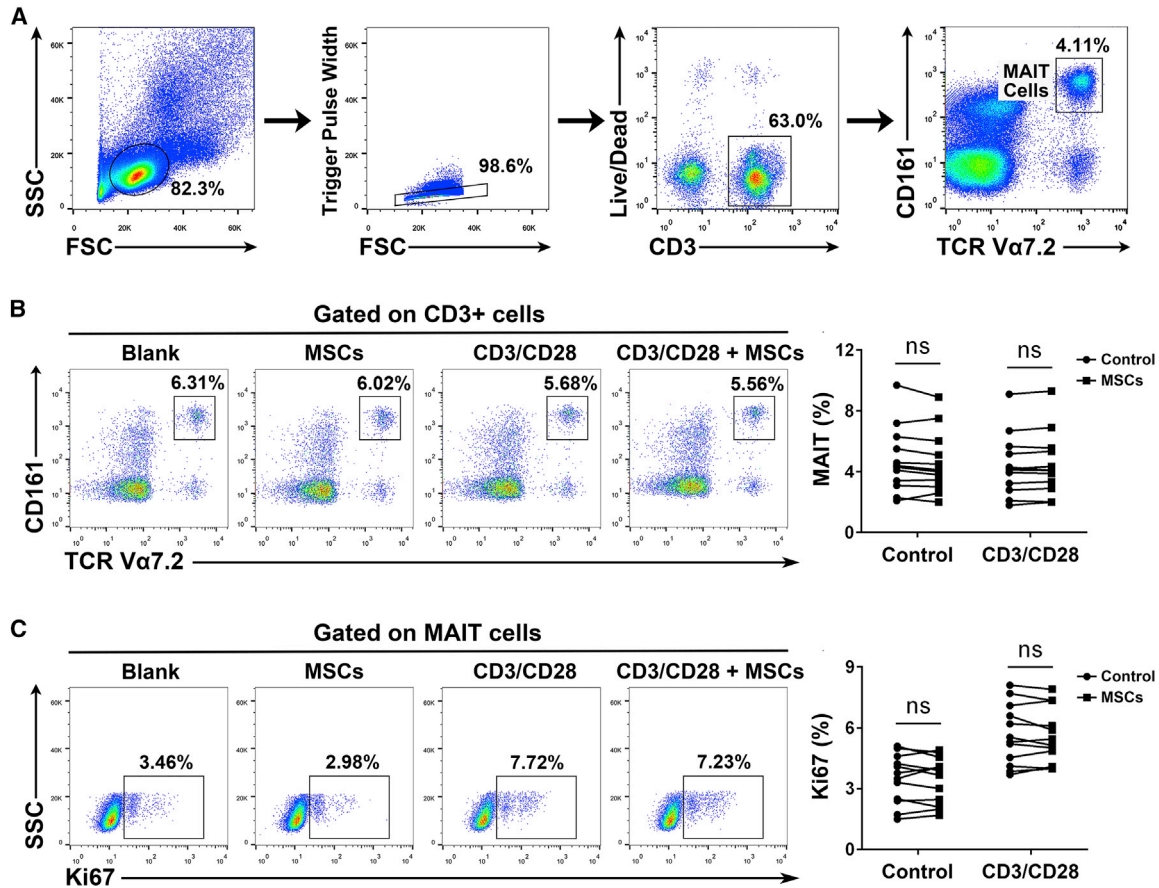


Figure 1. MSCs Do Not Alter the Proliferation of MAIT Cells

(A) MAIT cells were gated for several processes using flow cytometry.

(B) PBMCs were cultured individually or with MSCs for 3 days in the presence or absence of CD3/CD28 antibodies, and the percentages of MAIT cells among CD3+ T cells were analyzed. Regardless of whether CD3/CD28 antibodies were used, MSCs did not obviously alter the percentages of MAIT cells.

(C) Isolated MAIT cells were cultured individually or with MSCs for 3 days in the presence or absence of CD3/CD28 antibodies. Regardless of the presence of CD3/CD28 antibodies, MSCs did not alter the expression of Ki67 in MAIT cells. n = 12 independent experiments, ns, not significant.

RESULTS

MSCs Did Not Alter the Percentage and Proliferation of MAIT Cells

First, we investigated whether MSCs affect the proportion of MAIT cells among peripheral blood mononuclear cells (PBMCs). The MSCs we used were isolated from human bone marrow (BMMSCs), and the characteristics of MSCs are presented in [Figure S1](#). MAIT cells were defined as CD3+ TCR V α 7.2+ CD161+ cells ([Figure 1A](#)). We cultured PBMCs alone or with MSCs and found no significant difference in the proportions of MAIT cells among PBMCs, regardless of whether CD3/CD28 antibodies were used ([Figure 1B](#)). In addition, we separated MAIT cells from PBMCs using flow sorting and cultured MAIT cells alone or with

MSCs. Then, we compared the proliferative ability of MAIT cells by measuring the levels of Ki67, a common marker of cellular proliferation, and found that MSCs did not alter the percentage of Ki67+ MAIT cells ([Figure 1C](#)). Taken together, these data reveal that MSCs do not affect MAIT cell proliferation.

MSCs Could Activate MAIT Cells

Next, we wondered whether MSCs regulate the activation of MAIT cells. First, we stimulated MAIT cells in a traditional manner with *Escherichia coli*-infected monocytes, and the results showed that classically activated MAIT cells expressed CD69, TNF- α , IFN- γ , IL-17, and GB at high levels ([Figure S2](#)). To determine whether MSCs influence the activation of MAIT cells, we first detected the expression of



CD69, a marker of activation (Corbett et al., 2014), and found that, compared with MAIT cells cultured alone, MAIT cells cultured with MSCs expressed CD69 at higher levels, particularly after stimulation with CD3/CD28 antibodies (Figure 2A). Furthermore, we measured the expression of functional cytokines in MAIT cells and discovered that MSCs enhanced the expression of TNF- α , IFN- γ , and GB, but not IL-17, in MAIT cells (Figures 2B–2E). Consistent with the CD69 results, the upregulation of TNF- α , IFN- γ , and GB was enhanced after stimulation with the CD3/CD28 antibodies. In addition, we performed enzyme-linked immunosorbent assays (ELISAs) on the supernatant of MAIT cells and the results showed that, after coculture with MSCs, MAIT cells secreted TNF- α , IFN- γ , and GB at higher levels (Figure 2F).

To evaluate whether MSCs derived from other tissues exert the same effect on MAIT cells, we isolated umbilical cord blood-derived MSCs (UBMSCs) and adipose-derived MSCs (ADMSCs). The characteristics of UBMSCs and ADMSCs are presented in Figure S1. We cocultured MAIT cells with UBMSCs and ADMSCs separately, and the flow cytometry results showed that both UBMSCs and ADMSCs exerted a similar effect on MAIT cells to BMSCs (Figure S3). Then, we also cocultured MSCs with MAIT cells isolated from the liver or intestine and obtained similar results (Figure S4), indicating that MAIT cells derived from specific organs were also activated by MSCs. Furthermore, we also cocultured PBMCs with MSCs to test whether these effects of MSCs still existed when MAIT cells were not separated from PBMCs, and observed a similar effect of MSCs on activating MAIT cells (data not shown). Taken together, MSCs induce an activated phenotype of MAIT cells characterized by increased expression of CD69, TNF- α , IFN- γ , and GB.

MSCs Colocalized with MAIT Cells in Live Tissues

We performed immunofluorescence staining on sections of human bone marrow, liver tissue, and intestinal tissue to evaluate whether MSCs and MAIT cells interact in live tissues. MSCs were identified by staining with the CD105 antibody and MAIT cells were labeled as TCR V α 7.2+ cells. The results showed the colocalization of MSCs and MAIT cells in the sections of all these tissues (Figure 3), indicating that MAIT cells and MSCs interacted in live tissue and supporting the hypothesis that MSCs regulate MAIT cells in specific tissues.

MSCs Activated MAIT Cells in a TCR-MR1-Independent Manner

MAIT cells can be activated by cell-cell contact through a TCR-MR1 pathway or a TCR-MR1-independent pathway with cytokines (Le Bourhis et al., 2011). We first examined whether MSCs activated MAIT cells in a TCR-MR1-dependent manner to explore the mechanism by which MSCs

activate MAIT cells. Initially, we examined the expression of MR1 in MSCs using flow cytometry and found that MSCs expressed similar levels of MR1 as THP-1 cells, which have been reported to express MR1 (Laugel et al., 2016) (Figure 4A). Then, we blocked the TCR-MR1-dependent pathway using an MR1 antibody, but MR1 blockade did not alter the upregulation of CD69, TNF- α , IFN- γ , and GB in MAIT cells activated by MSCs (Figure 4B). These results indicate that an MR1-independent mechanism mediates MAIT cell activation by MSCs. To further verify this hypothesis, we cocultured MAIT cells with MSCs in Transwell plates. In the absence of cell-cell contact, MSCs induced MAIT cells to express increased levels of CD69, TNF- α , IFN- γ , and GB, but not IL-17 (Figure 4C). Moreover, we collected the supernatant produced by MSCs, and the supernatants were added to MAIT cells. Compared with the blank control supernatant, the supernatant produced by MSCs increased the expression of CD69, TNF- α , IFN- γ , and GB in MAIT cells (Figure S5), which further supported the hypothesis that MSCs activate MAIT cells in a cytokine-dependent manner. In summary, these findings show that MSCs activate MAIT cells in a TCR-MR1-independent manner.

MSCs Activated MAIT Cells by Secreting IL-15

In addition to the TCR-MR1-dependent mechanism, MSCs activated MAIT cells by secreting cytokines. Previous studies have reported that MAIT cells could be activated by IL-12, IL-18, IL-7, IL-15, IL1- β , and IFN- α (Chiba et al., 2012; Gracey et al., 2016; Li et al., 2019b). To identify the functional factors, we examined the mRNA expression of these cytokines in MSCs cultured alone or cocultured with MAIT cells using qPCR, with IL-6, IL-10, and TGF- β serving as controls. MSCs expressed IL-6 and TGF- β at high levels, but rarely expressed IL-10 (Figure 5A), consistent with previous reports. Critically, MSCs expressed moderate levels of IL-7 and IL-15, but not IL-12, IL-18, IL1- β , or IFN- α (Figure 5A).

Next, we added recombinant IL-6, IL-7, IL-15, and TGF- β separately or in combination to MAIT cells to evaluate whether these cytokines were sufficient to activate MAIT cells, and the results showed that IL-7 and IL-15 activated MAIT cells both alone and in combination (Figure S6). Then, we measured the concentrations of IL-7 and IL-15 in MSC supernatants using ELISAs, and the results showed that MSCs produced these two cytokines (Figure 5B). In addition, compared with MSCs cultured alone, the expression of IL-15 was upregulated in MSCs cocultured with MAIT cells (Figures 5A and 5B). Furthermore, we separately blocked IL-7 and IL-15 with neutralizing antibodies to assess the roles of IL-7 and IL-15 in MAIT cell activation by MSCs, and the results showed that blocking IL-15 almost completely reversed the increases in CD69, TNF- α ,

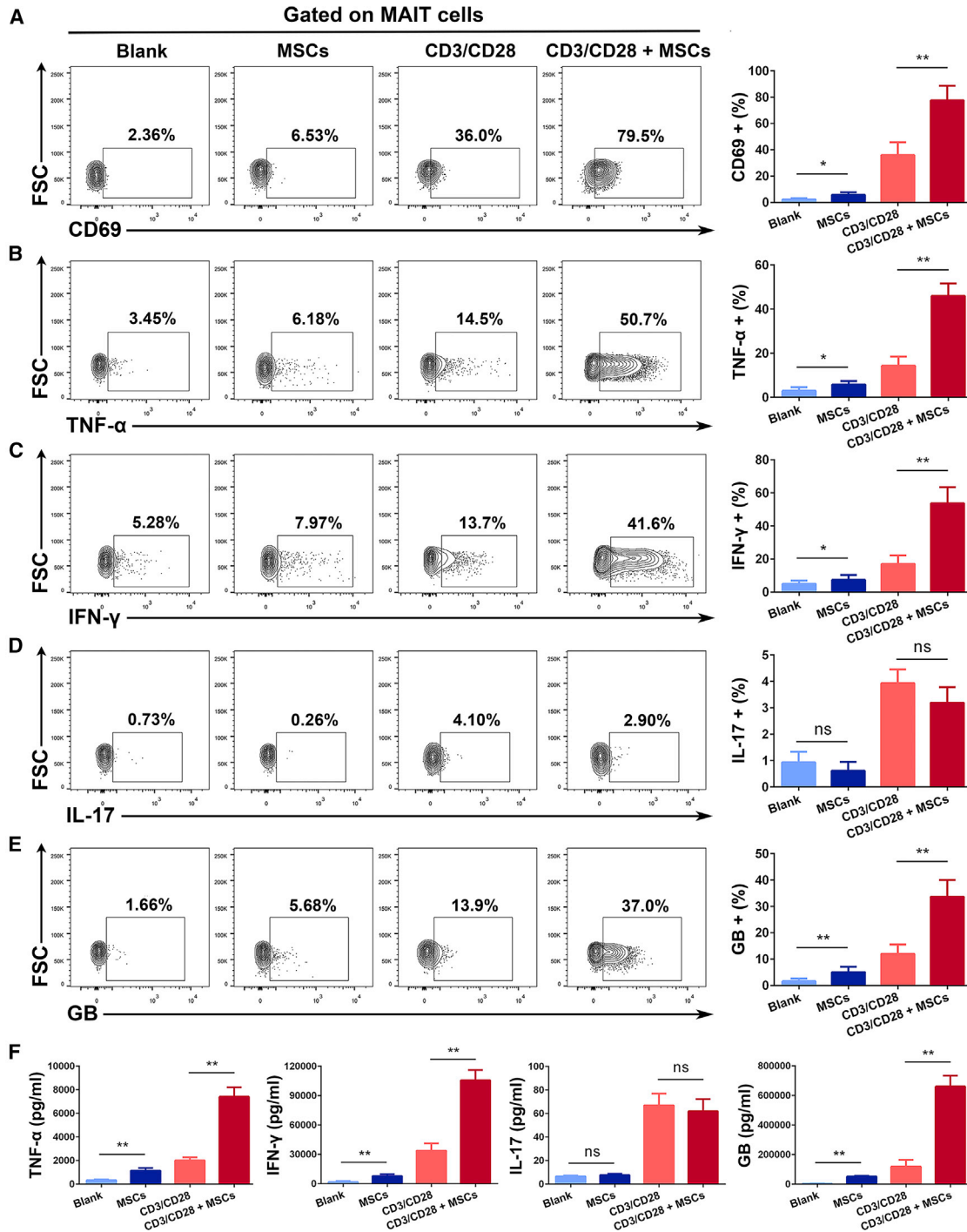


Figure 2. MSCs Activate MAIT Cells

Isolated MAIT cells were cultured individually, with MSCs, with CD3/CD28 antibodies or with CD3/CD28 antibodies plus MSCs for 2 days, and the levels of an activation marker and functional cytokines were measured.

(A–E) Compared with the cells cultured alone, the MAIT cells cultured with MSCs expressed CD69 (A), TNF- α (B), IFN- γ (C), and GB (E) at higher levels, but IL-17 levels were not altered (D). Compared with the cells cultured with CD3/CD28 antibodies, the MAIT cells cultured with CD3/CD28 antibodies plus MSCs exhibited obviously increased expression of CD69 (A), TNF- α (B), IFN- γ (C), and GB (E), but not IL-17 (D). (F) The ELISAs on the supernatant of MAIT cells showed that, after coculture with MSCs, MAIT cells secreted TNF- α , IFN- γ , and GB at higher levels but not IL-17. n = 12 independent experiments; *p < 0.05, **p < 0.01; ns, not significant.

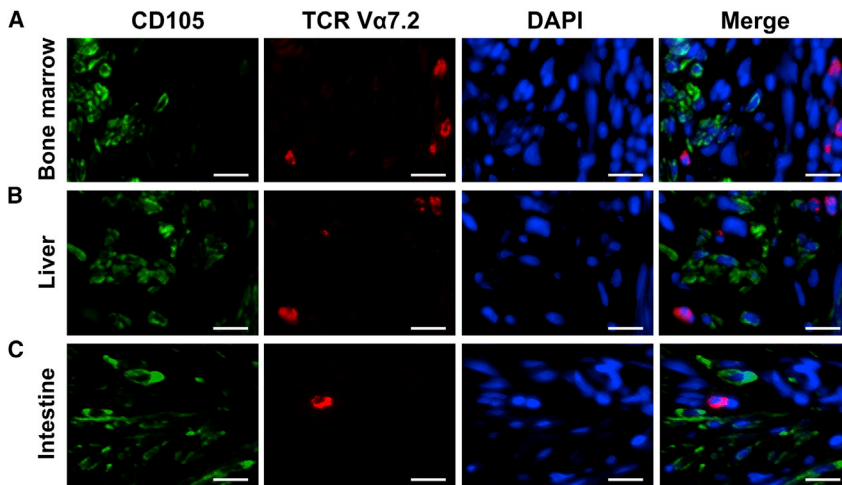


Figure 3. MSCs Colocalized with MAIT Cells in Live Tissues

Immunofluorescence staining showing the colocalization of CD105+ cells that represent MSCs and TCR Va7.2+ cells that represent MAIT cells in sections of human bone marrow (A), liver tissue (B), and intestinal tissue (C). n = 3 independent experiments. Scale bars, 10 nm.

IFN- γ , and GB expression in MAIT cells activated by MSCs, while blocking IL-7 had no apparent effect (Figures 5C–5F). We also downregulated the expression of IL-7 and IL-15 in MSCs using siRNAs to further verify these results, and we obtained parallel outcomes (Figures S7A–S7F). Furthermore, we also blocked IL-6 and TGF- β with neutralizing antibodies, and these two factors had no effect on MAIT cell activation (Figures S7G–S7J). Taken together, our data indicate that MSCs activate MAIT cells by secreting IL-15.

MSCs Facilitated Autophagy in MAIT cells through IL-15

Given that autophagy is involved in T cell activation and could be regulated by IL-15 (Bronietzki et al., 2015; Zhu et al., 2018), we investigated whether MSCs modulate the autophagy activity of MAIT cells. First, we performed Cyto-ID staining, which reportedly selectively stains autophagocytic vesicles (Chen et al., 2018) and found that, compared with MAIT cells cultured alone, MAIT cells cocultured with MSCs showed substantially increased Cyto-ID activity, as measured using flow cytometry (Figure 6A) and fluorescence microscopy (Figure 6B). Moreover, we detected the LC3-II/I ratio and p62 level and found that MSCs markedly increased the LC3-II/I ratio and reduced the p62 level in MAIT cells (Figure 6C). Furthermore, by blocking IL-15, we successfully rescued the aforementioned changes in MAIT cells activated by MSCs (Figures 6A–6C). Altogether, these results indicate that MSCs increase autophagy activity in MAIT cells through IL-15.

MSCs Activated MAIT Cells by Promoting Their Autophagy

Subsequently, we investigated whether MSCs activated MAIT cells by enhancing the autophagy activity of MAIT cells. First, we modulated the autophagy activity of MAIT

cells by treating them with the autophagy inducer rapamycin or the autophagy inhibitor 3-methyladenine (3-MA) (Cen et al., 2019). Rapamycin obviously increased the Cyto-ID activity and LC3-II/I ratio and decreased p62 expression in MAIT cells, whereas 3-MA had the opposite effects (Figures 7A–7C). Importantly, rapamycin and 3-MA increased and reduced the expression of CD69, TNF- α , IFN- γ , and GB, respectively, in MAIT cells (Figures 7D–7G). Subsequently, after the addition of 3-MA, the autophagy activity of MAIT cells cocultured with MSCs returned to the level of MAIT cells cultured alone (Figures 7A–7C). Furthermore, suppressing autophagy via 3-MA completely reversed the upregulation of CD69, TNF- α , IFN- γ , and GB in MAIT cells driven by MSCs (Figures 7D–7G). In summary, these findings reveal that autophagy regulates the activation of MAIT cells and MSC-activated MAIT cells by enhancing autophagy in MAIT cells.

DISCUSSION

In this study, we investigated whether MAIT cells are regulated by MSCs and the underlying mechanism. Our results reveal that MAIT cells develop an activated phenotype with increased expression of CD69, TNF- α , IFN- γ , and GB after exposure to MSCs. Mechanistically, IL-15 secreted by MSCs induces autophagy in MAIT cells, resulting in MAIT cell activation.

MAIT cells represent an important subtype of immune cells, comprising 1%–45% of T cells, and participate in many physiological and pathological processes (Debuschere et al., 2016; Toubal et al., 2019). MAIT cells are usually activated by antigen-presenting cells (APCs), such as dendritic cells or macrophages (Franciszkiwicz et al., 2016), but researchers have not examined whether MAIT cells are regulated by immunoregulatory cells. MSCs

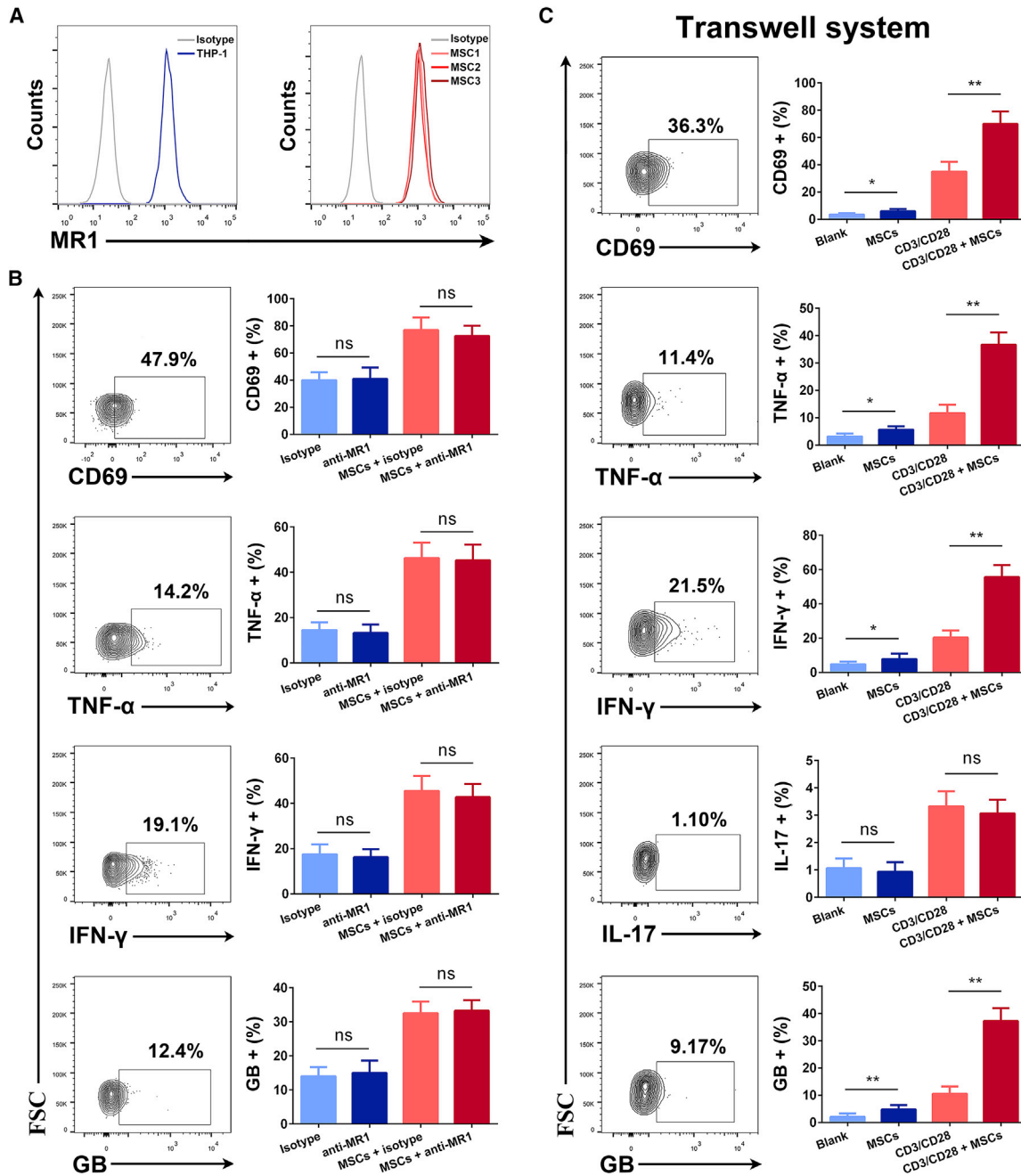


Figure 4. MSCs Activate MAIT Cells in a TCR-MR1-Independent Manner

(A) Flow cytometry detection showed that both THP-1 cells and MSCs expressed MR1.

(B) CD3/CD28 antibodies were added to the four groups, and the MAIT cells were cultured with or without MSCs and treated with an MR1 antibody or the isotype control for 2 days. Flow cytometry detection showed that the MR1 antibody did not alter the increased expression of CD69, TNF- α , IFN- γ , and GB in MAIT cells activated by MSCs.

(C) MAIT cells were cultured alone or with MSCs, CD3/CD28 antibodies, or CD3/CD28 antibodies plus MSCs in Transwell plates for 2 days, and the levels of an activation marker and functional cytokines were measured. Compared with the cells cultured alone, the MAIT cells cultured with MSCs expressed CD69, TNF- α , IFN- γ , and GB at higher levels, but the levels of IL-17 were not altered. Compared with the cells cultured with CD3/CD28 antibodies, the MAIT cells cultured with CD3/CD28 antibodies plus MSCs exhibited obviously increased expression of CD69, TNF- α , IFN- γ , and GB, but not IL-17. $n = 12$ independent experiments; * $p < 0.05$, ** $p < 0.01$; ns, not significant.

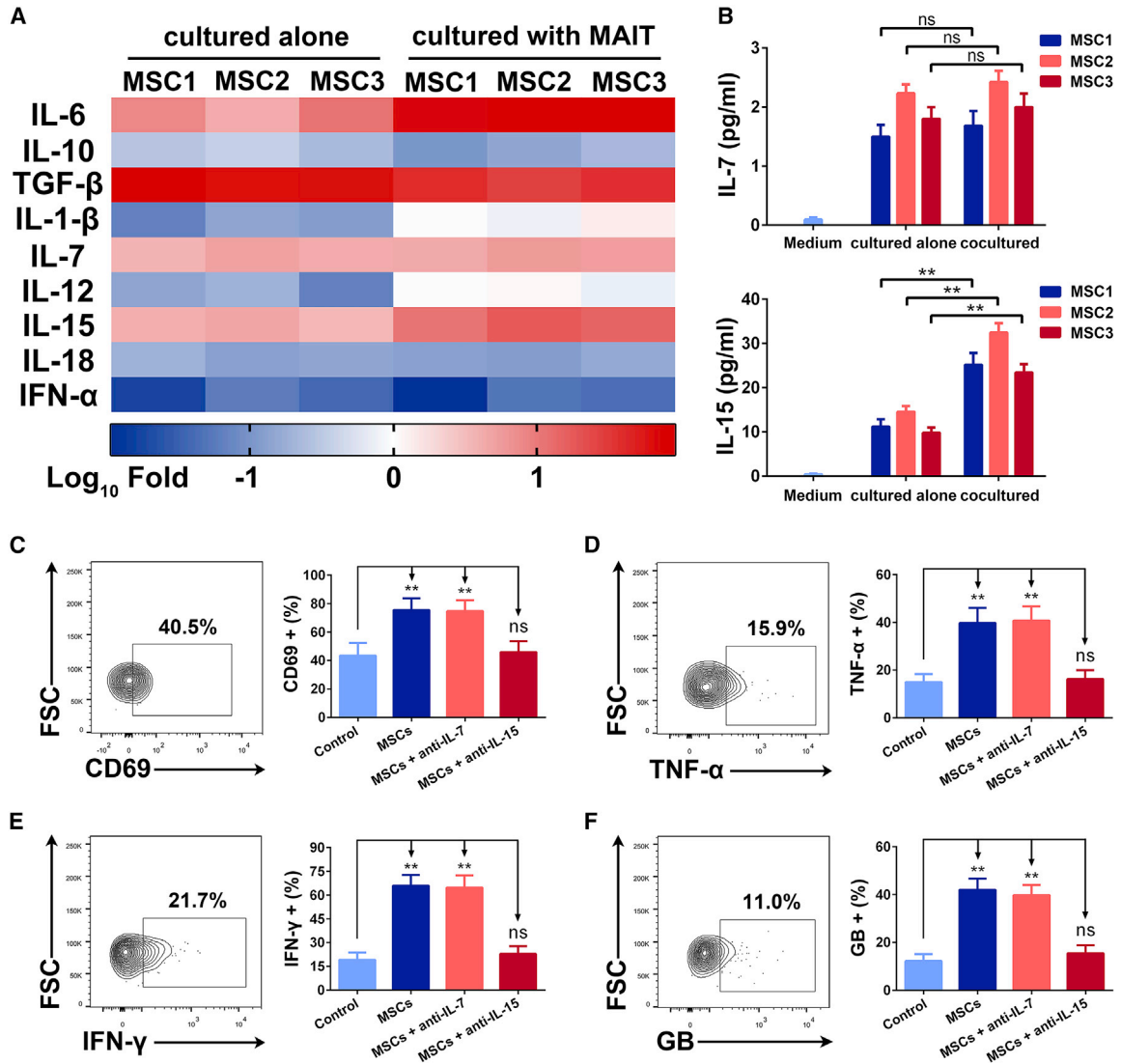


Figure 5. MSCs Activate MAIT Cells by Secreting IL-15

(A) The relative mRNA expression of several cytokines in MSCs was measured using quantitative real-time PCR. MSCs expressed IL-6, TGF-β, IL-7, and IL-15 at high levels, but not IL-10, IL-12, IL-18, IL1-β, or IFN-α. MSCs cocultured with MAIT cells expressed higher levels of IL-15 than MSCs cultured alone.

(B) ELISA detection of the culture supernatants from MSCs showed that MSCs secreted IL-7 and IL-15. MSCs cocultured with MAIT cells expressed higher levels of IL-15, but not IL-7, than MSCs cultured alone.

(C–F) All groups of MAIT cells were cultured with CD3/CD28 antibodies. IL-15 blockade reversed the increases in CD69 (C), TNF-α (D), IFN-γ (E), and GB (F) expression in MAIT cells activated by MSCs, while IL-7 blockade did not exert an obvious effect (C–F). n = 12 independent experiments; **p < 0.01; ns, not significant.

possess strong immunomodulatory properties and exert extensive effects on various types of immune cells (Glenn and Whartenby, 2014). For instance, MSCs inhibit the activation of NK cells (Sotiropoulou et al., 2006) and shift macrophage differentiation from an inflammatory M1 phenotype to an anti-inflammatory M2 phenotype (Abumaree et al., 2013). In addition, MSCs regulate the proliferation, differentiation, and function of T and B cells (Fan

et al., 2016; Keating, 2008). Here, we first showed the effect of MSCs on MAIT cells and found that the proliferation of MAIT cells was not altered by MSCs, but that the activation of MAIT cells was facilitated by MSCs. These findings helped determine the regulatory network of MAIT cells and the modulatory effect of MSCs on the immune system.

Since MSCs always exert an anti-inflammatory effect on the immune system and numerous studies, including our

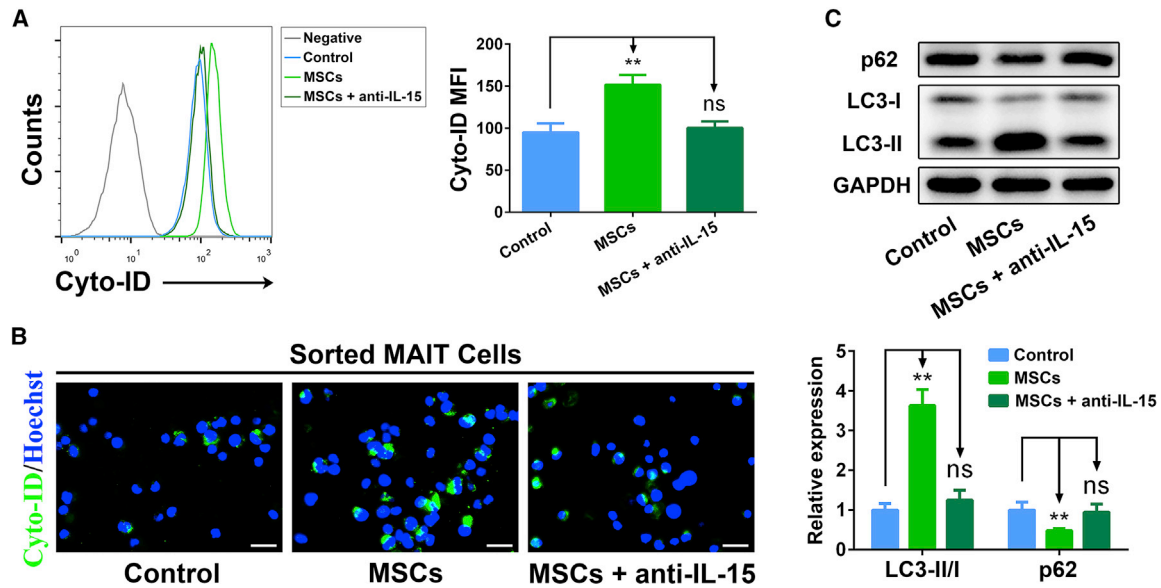


Figure 6. MSCs Induced Autophagy in MAIT Cells through IL-15. MAIT Cells Were Cultured with CD3/CD28 Antibodies Alone or in Combination with MSCs for 2 Days

(A and B) The mean fluorescence intensity (MFI) of Cyto-ID detected using flow cytometry (A) and the fluorescence signal of Cyto-ID observed under a fluorescence microscope (B) in the MAIT cells cocultured with MSCs were stronger than in the MAIT cells cultured alone, and IL-15 blockade abolished these differences.

(C) Western blot analysis showing that MSCs increased the LC3-II/I ratio and reduced the p62 level in MAIT cells, but IL-15 blockade abrogated these effects. $n = 12$ independent experiments; $**p < 0.01$; ns, not significant. Scale bars, 20 nm.

previous studies, have shown that MSCs inhibit various types of T cells (Li and Hua, 2017), we were surprised to find that MSCs activated MAIT cells but did not exert a suppressive effect. We consider that MSCs exert their suppressive effect on T cells mainly by secreting soluble cytokines, such as prostaglandin E2 (PGE-2), TGF- β , IL-6, and IL-10 (Gebler et al., 2012), while MAIT cells represent a special subtype of T cells lacking receptors for PGE-2, TGF- β , IL-2, and IL-6 (Tang et al., 2013), which might prevent the repressive effect of MSCs. In addition, MAIT cells express high levels of receptors for IL-1- β , IL-7, IL-12, IL-15, and IL-18, and can be activated by these cytokines (Chiba et al., 2012; Gracey et al., 2016; Li et al., 2019b). In addition, MSCs have been reported to exert a pro-inflammatory effect and may play different roles in different states (Waterman et al., 2010). In our study, we found that, when cocultured with MAIT cells, MSCs expressed high levels of IL-15, which resulted in MAIT cell activation.

To date, MAIT cells have been shown to be activated by a TCR-MR1-dependent mechanism or a cytokine-dependent mechanism (Franciszkiwicz et al., 2016; Le Bourhis et al., 2011). After stimulation with vitamin B2 metabolites from various bacteria or viruses, APCs, including dendritic cells and macrophages, induce an activated phenotype in MAIT cells in a TCR-MR1-dependent manner (Franciszkiwicz et al., 2016). MSCs have been shown to function as

APCs (Stagg et al., 2006), and our study showed that MSCs expressed MR1. However, MR1 blockade did not alter the effect of MSCs on MAIT cell activation, and MSCs still activated MAIT cells in the Transwell system. Based on these results, MSCs do not activate MAIT cells through a TCR-MR1 mechanism, at least in our experimental system. However, given that activation via TCR-MR1 requires vitamin B2 metabolites, which are usually derived from microbes, and we did not add the related metabolites or microbes to our experimental system, we cannot exclude the possibility that MSCs regulate MAIT cells through TCR-MR1 under certain conditions. Further investigations are needed to clarify this issue.

Regarding the TCR-MR1-independent pathway, several cytokines, including IL-7, IL-12, IL-15, and IL-18, could activate MAIT cells (Li et al., 2019b; Tang et al., 2013). In our study, we found that MSCs expressed IL-15 and that neutralizing or interfering with IL-15 successfully counteracted the activation of MAIT cells by MSCs. IL-15 is a crucial immunoregulatory factor that is widely expressed in various cell types (Steel et al., 2012) and has been reported to activate MAIT cells (Li et al., 2019b; Sattler et al., 2015). In addition, previous studies have reported that MSCs could express IL-15 and that MSC-derived IL-15 promoted the proliferation and activation of lymphocytes (Jing et al., 2014; Silva et al., 2003), consistent with our results.

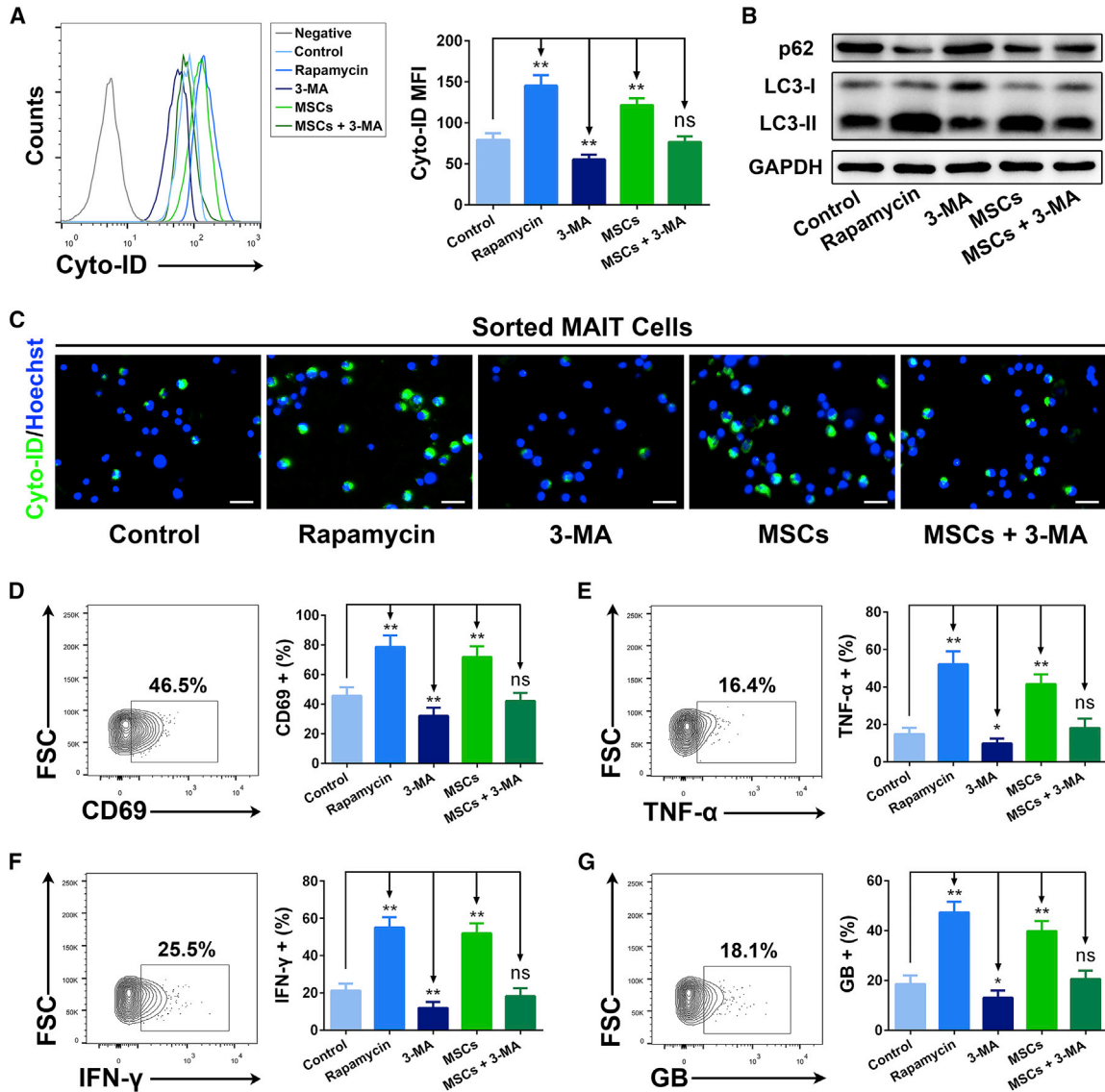


Figure 7. MSCs Activated MAIT Cells by Promoting Autophagy in MAIT Cells

All groups of MAIT cells were cultured with CD3/CD28 antibodies and received the corresponding treatments for 2 days. (A–C) Rapamycin and MSCs increased the MFI of Cyto-ID detected using flow cytometry (A), the fluorescence signal of Cyto-ID detected under a fluorescence microscope (B), and the LC3-II/I ratio and reduced the p62 level (C) in MAIT cells. The 3-MA treatment exerted the opposite effect and rescued the changes induced by MSCs (A–C). (D–G) Rapamycin upregulated and 3-MA downregulated the expression of CD69 (D), TNF-α (E), IFN-γ (F), and GB (G) in MAIT cells. The 3-MA treatment abrogated the increases in CD69 (D), TNF-α (E), IFN-γ (F), and GB (G) expression in MAIT cells induced by MSCs. n = 12 independent experiments; *p < 0.05, **p < 0.01; ns, not significant. Scale bars, 20 nm.

Altogether, these findings indicate that IL-15 plays an important role in the immunomodulatory function of MSCs.

Several studies have reported that IL-15 activates MAIT cells, but knowledge regarding the underlying mechanism is limited. Here, we reveal that MSC-derived IL-15 activates MAIT cells by inducing autophagy in MAIT cells. IL-15 has

been shown to regulate lymphocytes through the PI3K/mTOR pathway (Patidar et al., 2016), and Min-Seok recently showed that IL-15 activates hepatic CD8+ MAIT cells through the PI3K/mTOR pathway, which is a vital pathway involved in autophagy (Rha et al., 2020). In addition, IL-15 has been shown to maintain the survival of NKT cells by stimulating autophagy (Zhu et al., 2018), and IL-15



induced autophagy in liver-resident CD8⁺ T cells, which contain high proportions of MAIT cells (Swadling et al., 2020). Based on these results, IL-15 plays an important role in regulating autophagy and supports our findings suggesting that IL-15 activates MAIT cells by enhancing autophagy.

Autophagy is a vital process that is regulated by multiple factors and modulates various cellular functions (Brosnietzki et al., 2015). Rapamycin and 3-MA are a widely used activator and inhibitor of autophagy, respectively (Cen et al., 2019). After treatment with rapamycin or 3-MA, we successfully induced or suppressed autophagy activity in MAIT cells following stronger or weaker activation of MAIT cells, respectively. Previously, autophagy has been shown to be actively regulated in response to T cell activation (Hubbard et al., 2010), and increased autophagy levels were associated with higher expression of CD69, IFN- γ , and GB in CD8⁺ T cells (Swadling et al., 2020), indicating that autophagy is critical for the function of T cells, consistent with our findings. In contrast, here, we first explored the function of autophagy in MAIT cell activation and revealed that autophagy upregulates the expression of CD69, TNF- α , IFN- γ , and GB in MAIT cells. In addition, previous researchers have studied the effect of MSCs on T cell autophagy, but the results were inconsistent. Martin Böttcher and colleagues showed that bone marrow-derived MSCs fostered a T cell phenotype with increased autophagy (Böttcher et al., 2016), while Chen et al. (2016) reported that UBMSCs suppressed autophagy in T cells from patients with systemic lupus erythematosus (SLE). In our study, we found that MSCs enhance autophagy in a specific subtype of T cells, i.e., MAIT cells. Thus, the role of MSCs in T cell autophagy may vary, depending on the source of MSCs and T cells and the subtype of T cells.

MAIT cells represent an abundant subtype of T cells that play a crucial role in resisting microbial infections (Meermeier et al., 2018), and MSCs also reportedly exert an anti-infection effect (Alcayaga-Miranda et al., 2017; Rabani et al., 2018). In addition, both MAIT cells and MSCs have been reported to engage in many inflammatory or immune diseases, such as SLE and RA (Chiba et al., 2018; Pistoia and Raffaghello, 2017). Here, we reveal an interaction between MAIT cells and MSCs. We presume that the interaction between MAIT cells and MSCs may play an important role in some physiological and pathological processes and deserves further investigation.

In conclusion, we are the first group to report an interaction between MAIT cells and MSCs in which MAIT cells are activated by MSCs through IL-15-mediated autophagy and the first to reveal the regulatory effect of autophagy on MAIT cell activation. These findings may help elucidate the mechanisms underlying some immune responses and diseases and provide guidance for future research.

EXPERIMENTAL PROCEDURES

Ethics Statement

This study was approved by the Ethics Committee of The Eighth Affiliated Hospital, Sun Yat-sen University, Shenzhen, China. Written informed consent for the experimental requirements and potential risks was provided by all donors of peripheral blood and bone marrow.

Isolation of PBMCs and MAIT Cells

Peripheral blood was collected from healthy donors, and PBMCs were isolated from peripheral blood using Ficoll-Hypaque density gradient centrifugation (GE Healthcare Life Sciences) according to the manufacturer's protocol. Three liver tissues and three intestinal tissues were obtained from patients who underwent a relevant resection operation and PBMCs were isolated as described previously. For the separation of the MAIT cells, PBMCs were incubated with fluorescein isothiocyanate (FITC) anti-human CD3 (BD Pharmingen, 561807), APC-CY7 anti-human TCR V α 7.2 (BioLegend, 351714), or PE anti-human TCR V α 7.2 (BioLegend, 351706), and PE-CY5 anti-human CD161 (BD Pharmingen, 551138) antibodies. The MAIT cells were identified as CD3⁺ TCR V α 7.2⁺ CD161⁺ cells and isolated using a flow sorter (BD Influx) according to the manufacturer's instructions. The isolated PBMCs or MAIT cells were directly used in subsequent experiments.

Isolation of MSCs

MSCs were isolated as described previously (Chen et al., 2020; Mahmoudifar and Doran, 2015; Xie et al., 2016). Twelve healthy volunteers were recruited for bone marrow puncture in the posterior superior iliac spine. Three fresh umbilical cords were obtained from mothers who provided informed consent, and three adipose tissue samples were obtained from patients undergoing liposuction surgery who provided informed consent. MSCs were immediately isolated by centrifugation at 2,500 \times g for 30 min. Then, MSCs were resuspended and cultured in culture flasks with Dulbecco's modified Eagle's medium (Gibco) containing 10% fetal bovine serum (Zhejiang Tianhang Biotechnology). After 2 days, the culture medium was replaced to deplete the suspended cells. Thereafter, the culture medium was replaced every 3 days. When the MSCs reached 80%–90% confluence, they were digested and divided into two new culture flasks. In addition, the MSCs were used at passage 4 for the experiments, and each relevant experiment was independently repeated three times.

MSC Identification

For the detection of surface markers, MSCs were stained with antibodies against human CD29, CD44, CD105, CD45, CD14, and HLA-DR (all from BD Pharmingen, 559883, 561858, 560819, 560178, 561385, and 561359, respectively) and then analyzed using flow cytometry. For the measurement of the differentiation potential, MSCs were cultured with adipogenic induction medium, osteogenic induction medium, or chondrogenic induction medium for 21 days and oil red O staining, alizarin red S staining, and Alcian blue staining, respectively, was performed to evaluate the ability of MSCs to differentiate into the corresponding cell types.



Cell Culture and Treatment

The isolated PBMCs or MAIT cells were cultured alone or cocultured with MSCs at a ratio of 5:1 (2.5×10^5 PBMCs: 0.5×10^5 MSCs in 12-well plates, 1.25×10^5 MAIT cells: 0.25×10^5 MSCs in 24-well plates) in RPMI-1640 medium containing 10% fetal bovine serum (Zhejiang Tianhang Biotechnology). For the Transwell coculture, 6.5 mm Transwell chambers with a 0.4 μ m pore polycarbonate membrane insert (Corning) were used, with MSCs seeded in the upper insert and MAIT cells seeded in the lower chamber. In addition, PBMCs or MAIT cells were cultured with or without stimulation with a purified CD3 antibody (1 μ g/mL, BD Pharmingen, 555336) and purified CD28 antibody (1 μ g/mL, BD Pharmingen, 555725). For the blocking experiments, 20 μ g/mL anti-human MR1 (BioLegend, 361103), 10 μ g/mL anti-human TGF- β (R&D Systems, AF-246-NA), 1 μ g/mL anti-human IL-6 (R&D Systems, MAB206-100), 10 μ g/mL anti-human IL-7 (BioLegend, 501304), 10 μ g/mL anti-human IL-15 (BioLegend, 515001), or the corresponding amount of isotype control (BioLegend, 400224) was added to the culture medium. For treatment with recombinant proteins, 20 ng/mL recombinant human TGF- β , 20 ng/mL recombinant human IL-6, 20 ng/mL recombinant human IL-7, and 50 ng/mL recombinant human IL-15 (all from R&D Systems) were used. Cells were treated with 500 nM rapamycin and 10 mM 3-MA to modulate autophagy.

MR1-Specific Activation of MAIT Cells

MAIT cells were activated as described previously (Leeansyah et al., 2013). *E. coli* strain D21 was cultured, washed with PBS, and fixed with 1% paraformaldehyde for 5 min. Monocytes were then administered PFA-fixed *E. coli* at an MOI of 50 for 6 h, followed by the addition of sorted MAIT cells. Cells were then cocultured for 1 day and collected for relevant measurements.

Flow Cytometry

PBMCs and MAIT cells were collected and suspended in PBS. Then, staining for the markers located on the cytomembrane and the Live/Dead labeling kit was performed for 30 min at room temperature. For the intracellular staining, the cells were further fixed for 15 min and washed with PBS. Then, the cells were permeabilized and stained with antibodies against intramembranous markers. Finally, the stained cells were detected using flow cytometry (BD FACVerse), and the results were analyzed using FlowJo v.10 software. In addition, for the detection of the secretory factors, a cell stimulation cocktail (plus protein transport inhibitors) containing phorbol 12-myristate 13-acetate (PMA), ionomycin, brefeldin A, and monensin (Invitrogen) was added to the culture medium 6 h before staining. The following antibodies were used for flow cytometry: FITC anti-human CD3 (BD Pharmingen, 561807), APC anti-human CD3 (BD Pharmingen, 561804), PE anti-human CD161 (BD Pharmingen, 556081), PE-Cy5 anti-human CD161 (BD Pharmingen, 551138), Alexa Fluor 488 anti-human Ki-67 (BD Pharmingen, 558616), PE-Cy7 anti-human CD69 (BD Pharmingen, 557745), PE anti-human TNF- α (BD Pharmingen, 554513), BV421 anti-human IFN- γ (BD Pharmingen, 562988), BV510 anti-human granzyme B (BD Pharmingen, 563388), Alexa Fluor 647 anti-human IL-17A (BD

Pharmingen, 560437), APC-Cy7 anti-human TCR V α 7.2 (BioLegend, 351714), PE anti-human TCR V α 7.2 (BioLegend, 351706), APC anti-MR1 (BioLegend, 361108), eFluor 660 fixable viability dye (Invitrogen, 65-0864-18), and eFluor 780 fixable viability dye (Invitrogen, 65-0865-18).

Immunofluorescence Staining

Fresh bone marrow, liver tissue, and intestinal tissue were deparaffinized and hydrated after fixation with formaldehyde. Then, deparaffinization and antigen repair were performed on the tissue sections. Afterward, the sections were blocked with 10% goat serum for 30 min and incubated with anti-CD105 (Abcam, ab231774) and anti-TCR V α 7.2 (BioLegend, 351702) antibodies at 4°C overnight. Then, the sections were stained with fluorophore-labeled secondary antibodies (Cell Signaling Technology, 4409S and 4412S), and 4',6-diamidino-2-phenylindole was used to stain cell nuclei. The sections were observed with a fluorescence microscopy according to the manufacturer's instructions.

Western blot Analysis

The experiment was performed as described previously (Li et al., 2019a). In brief, the cells were lysed with RIPA buffer (CW BIO) for 30 min on ice, and the lysates were collected and centrifuged at 14,000 rpm for 30 min at 4°C. Then, the protein samples were mixed with sodium dodecyl sulfate (SDS) loading buffer. Next, the proteins were separated via SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes (Millipore). After blocking with 5% non-fat milk for 1 h, the membranes were incubated with an anti-LC3 antibody (Santa Cruz, sc-398822), anti-p62 antibody (Cell Signaling Technology, 39749S), or anti-GAPDH antibody (CW BIO, CW0100M) at 4°C overnight. Finally, the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (Boster, BA1054 and BA1050) for 1 h and measured using the Immobilon Western Chemiluminescent HRP Substrate (Millipore).

RNA Extraction and Quantitative Real-Time PCR

RNA extraction and quantitative real-time PCR were performed as described previously (Li et al., 2018). In brief, RNA was extracted from MSCs using TRIzol (TaKaRa) and immediately reverse transcribed into cDNAs using PrimeScript RT Master Mix (TaKaRa). Then, SYBR Premix Ex Taq (TaKaRa) was used for quantitative real-time PCR, and gene expression was examined using a 7500 Real-Time PCR System (Applied Biosystems) according to the manufacturer's instructions. The relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method with GAPDH as the reference gene, and the relevant primers are listed in Table S1.

ELISA

The concentrations of TNF- α , IFN- γ , GB, IL-17, IL-7, and IL-15 in the cell culture supernatants were detected using the corresponding Quantikine ELISA kits (R&D Systems), according to the manufacturer's instructions. The absorbances of the reactants were measured using a Varioskan Flash Multimode Reader (Thermo Fisher), and the concentrations were determined from the standard curves generated with the standard substance.



RNA Interference and Transfection

Small interfering RNAs (siRNAs) targeting IL-7 and IL-15 and a negative control siRNA were purchased from GenePharma (Suzhou, China). The siRNA sequences are listed in Table S2. Transfections were performed with Lipofectamine RNAiMAX (Thermo Fisher) according to the manufacturer's protocol. In brief, MSCs were seeded and transfected with siRNAs and Lipofectamine RNAiMAX after reaching 70%–90% confluence. Then, the MSCs were collected to determine the interference efficiency after 48 h or directly used in the experiments.

Cyto-ID Autophagy Detection

A Cyto-ID Autophagy Detection Kit (Enzo Life Sciences) was used to measure autophagy in MAIT cells according to the manufacturer's protocols. In brief, after applying treatments according to the experimental requirements, MAIT cells were collected and stained with Cyto-ID Green dye alone or together with Hoechst 33342 at 37°C for 30 min in the dark. Then, the cells were washed and resuspended in assay buffer. Finally, the autophagy activity of the MAIT cells was detected using flow cytometry and fluorescence microscopy.

Statistical Analysis

The MSC-related experiments were conducted with MSCs from five different donors, and all experiments were performed in triplicate. All data were analyzed with SPSS 18.0 software and are presented as means \pm standard deviation (SD). Differences between two groups were compared with independent sample t tests. $p < 0.05$ was regarded as statistically significant.

Data and Code Availability

The data that support the findings of this study are available from the corresponding author upon request.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at <https://doi.org/10.1016/j.stemcr.2021.03.005>.

AUTHOR CONTRIBUTIONS

G.Y., P.W., Y.W., and H.S. designed the study. G.Y., Z.X., Q.C., and Z.L. performed the experiments. J.L., G.Z., and S.W. analyzed the data. G.Y., M.L., W.L., and S.C. wrote the manuscript. Z.X., W.Y., and Y.W. revised the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

ACKNOWLEDGMENTS

This study was financially supported by the National Natural Science Foundation of China (81871750 and 81971518), the Key Realm R&D Program of Guangdong Province (2019B020236001), the Science and Technology Project of Shenzhen City (ZDSYS20190902092851024), and the Fundamental Research Funds for the Central Universities (19ykpy01).

Received: August 4, 2020

Revised: March 2, 2021

Accepted: March 3, 2021

Published: April 13, 2021

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