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ODC1 inhibits the inflammatory response and ROS-induced apoptosis in macrophages

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ABSTRACT

Macrophage activation plays a critical role in the innate immune response. Ornithine decarboxylase (ODC1) metabolizes L-ornithine to polyamines and is the rate-limiting enzyme involved in the metabolism of polyamines, which are reportedly involved in cell differentiation, proliferation, and migration. However, the function of ODC1 in immune cells and especially in macrophages, as well as its underlying molecular mechanism, remains unclear. This study revealed the potential ODC1 function and mechanism associated with the lipopolysaccharide (LPS)-, *Bacillus Calmette–Guerin* (BCG)-, or carbon tetrachloride (CCl_4)-induced inflammatory response in macrophages. We found significant upregulation of ODC1 in macrophages following LPS simulation and ODC1-specific suppression of proinflammatory cytokine secretion from macrophages upon stimulation with LPS, BCG and CCl_4, respectively, suggesting a role as a common control element of the inflammatory response. Western blotting for nuclear factor-κB and mitogen-activated protein kinases revealed significant inhibition of phosphorylation of multiple transcription factors following ODC1 overexpression in macrophages. Moreover, ODC1 inhibited reactive oxygen species-induced and caspase-dependent apoptosis highlighted by decreased caspase-3 and -9 expression following ODC1 upregulation. These findings indicated that ODC1 was involved in attenuating the inflammatory response upon stimulation of macrophages, making it a potential therapeutic target for inflammatory diseases.

1. Introduction

Polyamines, including putrescine, spermidine, and spermine, are a group of bioactive aliphatic compounds widely found in all organisms. Polyamine metabolism is involved in cell proliferation, differentiation, and other important biological processes through the regulation of gene expression [1–3]. Ornithine decarboxylase (ODC1) is the first rate-limiting enzyme in the polyamine-biosynthesis pathway [4], and crystallographic structures show that ODC1 comprises two domains in both human and mouse species [5,6]. As a homodimer, ODC1 decarboxylates L-ornithine to form putrescine, which is subsequently converted into spermidine and spermine [3].

ODC1 is induced in the liver of mice within hours of injection of various inflammatory agents, with lipopolysaccharide (LPS) being the most potent inducer [7]. Additionally, a previous study reported ODC1 induction in macrophages by bacterial LPS and mycobacterial cell-wall material [8], and another study showed that asparagine, an ODC1 activator, mitigated LPS-induced injury of the intestinal structure and barrier function by regulating inflammatory signaling pathways [9]. Chronic inflammation can promote cancer progression, with a number of studies documenting that rapid proliferation of tumor cells is dependent upon elevated ODC1 activity and polyamine levels [10,11] in some cancers, including gastric, colorectal [12,13], prostate [12,14], and breast [3,15]. These findings suggest that ODC1 might function as a regulator of the inflammatory response.

Macrophages play a critical role in host immune response following biological stimulation, bacterial infection, or external damage [16,17]. Macrophages are predominantly polarized into two types under different physiological and disease states [i.e., classically activated macrophages (M1 macrophages) and alternatively activated macrophages (M2 macrophages)] that participate in a variety of inflammatory processes [18,19]. Myeloid-specific ODC1 deletion significantly increased gastric and colonic inflammation

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caused by *Helicobacter pylori* infection, whereas reinstatement of putrescine, an ODC1 product, reversed the associated increase in M1 macrophage activation, indicating that ODC1 and putrescine are regulators of macrophage function [20]. However, the underlying mechanism associated with macrophage-specific ODC1 in regulating the inflammatory response remains unclear.

Here, we examined the role of macrophage-specific ODC1 in inflammation following activation by various stimuli, including LPS, *Bacillus Calmette –Guerin* (BCG), and carbon tetrachloride (CCl_4). Our findings showed that *ODC1* expression was significantly upregulated in macrophage cell lines and bone-marrow derived macrophages (BMDMs) upon stimulation, and that the inflammatory response induced by LPS, BCG, or CCl_4 could be significantly suppressed in an ODC1-specific manner via nuclear factor (NF)-κB and mitogen-activated protein kinase (MAPK) signaling. Furthermore, we found that ODC1 inhibited macrophage apoptosis by decreasing reactive oxygen species (ROS) generation following stimulation. Our findings provide insight into ODC1-mediated regulation of macrophage-dependent inflammation and suggest it as a potential therapeutic target in inflammation diseases.

2. Materials and methods

2.1. Cell and BCG culture

The murine macrophage cell line RAW264.7 was cultured in Dulbecco's modified Eagle medium (Hyclone, USA), BMDMs were isolated from the tibias and femurs of C57BL/6 (6–8-week old), BCG (ATCC35733; ATCC, Manassas, VA, USA) was grown in Middlebrook 7H9 broth medium supplemented with 10% OADC (BD Biosciences, San Diego, CA, USA). The method for cell culture is described in more detail in Supplementary Material.

2.2. Statistical analysis

Data were expressed as the mean ± standard deviation and analyzed using the Student's *t*-test. A $p < 0.05$ was considered statistically significant. All experiments were performed independently and repeated at least three times.

The more detailed Material and methods about Reagents and antibodies, Generation of plasmids and stable transfected cells, Quantitative PCR (qPCR), ELISA, Immunoblotting and Measurement of ROS by flow cytometry, are described in supplementary Material and methods.

3. Results

3.1. ODC1 regulates inflammatory cytokine expression in macrophages upon LPS stimulation

Previous studies demonstrated that ODC1 activity in rat colonic mucosa was enhanced during dimethylhydrazine-induced carcinogenesis [21], and that ODC1 represents a sensitive indicator of inflammatory derangement of the mucosa, especially in acute ulcerative colitis [22]. In the present study, we determined whether ODC1 derived from macrophages is involved in the macrophage-specific inflammatory immune response. We found that ODC1 expression in RAW264.7 cells increased during the first 24 h following stimulation, followed by a reduction after 36 h (Fig. 1A). Moreover, we observed increased ODC1 expression within 48 h following stimulation of BMDMs (Fig. 1B). To investigate the effect of ODC1 on macrophage-mediated inflammatory response, we established a cell line stably overexpressing ODC1 (RAW264.7-ODC1) and another exhibiting downregulated ODC1 expression (shRNA-ODC1) in RAW264.7 cells. Western blotting confirmed the respective changes in ODC1 level in each cell line (Fig. 1C and D), and subsequent measurement of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 secretion in the supernatant of shRNA-ODC1 cells following LPS treatment revealed significant increases in cytokine levels relative to those in control cells (Fig. 1E). Furthermore, we consistently observed decreased IL-1β and IL-6 secretion in RAW264.7-ODC1 cells as compared with that in RAW264.7-Vector (control) cells at 12 h post-stimulation with LPS (Fig. 1F). Real-time polymerase chain reaction (PCR) confirmed reduced cytokine levels at the mRNA level (Supplementary Fig. S1). These data demonstrated that macrophage-derived ODC1 might be involved in inhibiting the macrophage-induced inflammatory response upon LPS stimulation.

3.2. ODC1 mediates the inflammatory response

In addition to LPS stimulation, inflammation can also be induced upon other different stimulus. In the aspect of infection stimulation, we used the BCG vaccine derived from a strain of *Mycobacterium bovis* against tuberculosis due to its ability to survive within macrophages to promote a pulmonary inflammatory response [23,24]. Additionally, previous studies indicated that the hepatotoxic-chemical CCl_4 is capable of inducing liver injury accompanied by inflammation, apoptosis, or oxidative stress in animal models [25,26]. To investigate whether ODC1 regulates BCG- or CCl_4-induced inflammatory response in macrophages, RAW264.7-Vector and RAW264.7-ODC1 cells were treated with BCG or CCl_4 for 12 h, followed by measurement of inflammatory cytokine levels. We found that IL-1β and IL-6, but not TNF-α level, were significantly decreased in RAW264.7-ODC1 cells as compared with those in RAW264.7-Vector cells for both treatments (Fig. 2A and B), with associated decreases in mRNA levels confirmed by PCR (Supplementary Fig. S2). By contrast, ODC1 downregulation resulted in elevated levels of TNF-α, IL-1β, and IL-6 following stimulation as compared with those measured in control cells (Fig. 2C and D). These results were consistent with those obtained following LPS stimulation and suggested that ODC1 might suppress inflammatory responses triggered by different stimuli.

3.3. NF-κB and MAPK activation are impaired in ODC1-overexpressing macrophages

ODC1 is the first and rate-limiting enzyme involved in polyamine biosynthesis, with spermine the final product of polyamine metabolism [4]. Therefore, we added exogenous spermine to the culture medium of shRNA-ODC1 cells in order to investigate its effect on the enhanced inflammatory response induced by ODC1 downregulation. Unexpectedly, addition of spermine (5 μM) had no effect on cytokine expression relative to levels observed in shRNA-ODC1 cells stimulated by LPS (Fig. 3A) or BCG (Fig. 3B), implying that the effects of ODC1 on inflammation might not be dependent upon the polyamine-biosynthesis pathway.

Previous studies demonstrated that NF-κB and MAPK play critical roles in proinflammatory cytokine production [27,28]. Therefore, we examined whether ODC1 in macrophages regulates the inflammatory response via NF-κB and MAPK signaling. Western blot analysis of major transcription factors associated with NF-κB revealed that phosphorylation of p65 and IKK-α were significantly decreased at different time points in RAW264.7-ODC1 cells as compared with RAW264.7-Vector cells following LPS, BCG or CCl_4 treatment (Fig. 3C and Supplementary Fig. S3).

To obtain further insight into the molecular mechanisms by which ODC1 inhibits inflammation, phosphorylation of ERK1/2, p38, and stress-activated protein kinase/c-Jun NH2-terminal kinase (JNK) in the MAPK-signaling pathway were studied.

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