Taurine chloramine potentiates phagocytic activity of peritoneal macrophages through up-regulation of dectin-1 mediated by heme oxygenase-1–derived carbon monoxide

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ABSTRACT: Resolution of inflammation that occurs after microbial infection or tissue damage is an important physiologic process in maintaining or restoring host homeostasis. Taurine chloramine (TauCl) is formed by a reaction between taurine and hypochlorite in leukocytes, and it is especially abundant in activated neutrophils that encounter an oxidative burst. As neutrophils undergo apoptosis, TauCl is released to the extracellular matrix at the inflamed sites, thereby affecting coexisting macrophages in the inflammatory microenvironment. In this study, we investigated the role of TauCl in phagocytosis by macrophages during resolution of fungal infection–induced inflammation. We found that exogenous TauCl substantially increased the phagocytic efficiency of macrophages through up-regulation of dectin-1, a receptor for fungal β-1,3-glucans, which is present on the membrane of macrophages. Our previous studies demonstrated the induction of heme oxygenase-1 (HO-1) expression in murine peritoneal macrophages treated with TauCl. In the present study, knocking out HO-1 or pharmacologic inhibition of HO-1 with zinc protoporphyrin IX attenuated the TauCl-induced expression of dectin-1 and subsequent phagocytosis. Furthermore, carbon monoxide (CO), a by-product of the HO-1–catalyzed reaction, induced expression of dectin-1 and potentiated phagocytic capability of the macrophages, which appeared to be mediated through up-regulation of peroxisome proliferator–activated receptor γ. Taken together, induction of HO-1 expression and subsequent CO production by TauCl are essential for phagocytosis of fungi by macrophages. Our results suggest that TauCl has important roles in host defense against fungal infection and has therapeutic potential in the management of inflammatory diseases.—Kim, S. H., Zhong, X., Kim, W., Kim, K., Suh, Y.-G., Kim, C., Joe, Y., Chung, H. T., Cha, Y.-N., Surh, Y.-J. Taurine chloramine potentiates phagocytic activity of peritoneal macrophages through up-regulation of dectin-1 mediated by heme oxygenase-1–derived carbon monoxide. FASEB J. 32, 2246–2257 (2018). www.fasebj.org

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Detection of infected microbial pathogens and stimulation of innate immune responses by phagocytes are critical for maintaining or restoring host homeostasis (1–3).

Phagocytes, such as macrophages, have major roles in host defense against microbial infections by removing the pathogens through modulation of the immune responses. When microbial infection occurs, both macrophages and neutrophils are recruited to the inflamed site from circulating blood to detect, kill, and engulf the invading microorganisms. Among the various infections caused by different microbial species, fungal infections are prevalent because of increasing immunosuppressive medical treatment (4, 5).

Acute inflammatory response to fungal infection is accompanied by production of endogenous mediators (6) that are involved in resolution of acute inflammation (7, 8). Upon microbial infection, recruited neutrophils over-produce reactive oxygen species (ROS) and hypochlorous.
acid that protect the host from pathogens by oxidizing them. However, the overproduction of these reactive species may cause oxidative stress and chronic inflammation in host cells. Therefore, timely neutralization of these oxidants is required to maintain homeostasis (9, 10). Taurine, a semiessential, sulfur-containing β-amino acid, is present at high concentrations in most cells of all animal species (11). Notably, taurine, which is abundant in neutrophils, reacts stoichiometrically with hypochlorous acid to produce taurine chloramine (TauCl) (12). Once the activated neutrophils undergo apoptosis, TauCl then acts as a local autacoid to the abundant macrophages at the inflamed site. Although TauCl has bactericidal and fungicidal activity (13–15), the underlying molecular mechanisms are poorly understood.

To clear fungal infections, macrophages accumulated at the inflammation site must detect and engulf the fungi through phagocytic receptors that recognize fungal cell-specific surface molecules called pathogen-associated molecular patterns (PAMPs) (16). Dectin-1 is one of the pattern recognition receptors (PRRs) that recognize β-1,3-glucan, which constitutes the major cell wall of multiple pathogenic fungi, including Candida albicans. Dectin-1 has a central role in the host defense against fungal infection through peroxisome proliferator–activated receptor γ (PPAR-γ) activation (17). Upon recognition of fungal cell wall carbohydrates, the transmembrane receptor dectin-1 mediates phagocytosis of fungi and initiates an acute inflammatory response by producing inflammatory cytokines (18, 19). The loss of dectin-1 provokes devastating or aberrant immune response, which leads to an inability of macrophages to engulf fungi (20). Here, we report that TauCl could potentiate phagocytic activity of macrophages through up-regulation of dectin-1, mediated by the stress-responsive protein heme oxygenase-1 (HO-1) overexpression upon fungal infection.

MATERIALS AND METHODS

Materials

TauCl as a crystalline sodium salt (MW 181.57) was prepared according to previously published methods (21). DMEM, penicillin, streptomycin, and fetal bovine serum were obtained from Thermo Fisher Scientific (Waltham, MA, USA). Taurine, carbon monoxide–releasing molecule-3 (CORM-3), yeast malt broth, FITC, GW9662, hemoglobin (Hb), and antibodies against actin were purchased from MilliporeSigma (Billerica, MA, USA). Red blood cell lysis buffer was a product from iNTRON Biotechnology (Seongnam, South Korea). Curdlan was purchased from InvivoGen (San Diego, CA, USA). Primary antibodies against PPAR-γ and zinc protoporphyrin IX (ZnPP) were supplied by Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti–HO-1 was the product of Enzo Life Sciences (Farmingdale, NY, USA), and anti-rabbit and anti-mouse horseradish peroxidase–conjugated secondary antibodies were provided by Thermo Fisher Scientific. PVDF membranes were supplied by Pall Corp. (Port Washington, NY, USA). The ECL detection kit was obtained from GE Healthcare Life Sciences (Little Chalfont St. Giles, Buckinghamshire, United Kingdom).

Mice

C57BL/6 mice (6–9 wk old) were purchased from Central Lab Animal, Inc. (Seoul, South Korea). HO-1 knockout (KO) mice were provided by Dr. M. A. Perrella (Harvard Medical School, Boston, MA, USA). All the animals were maintained according to the institutional animal care guidelines. Animal experimental procedures were approved by the Institutional Animal Care and Use Committee at Seoul National University.

Cell culture

Primary peritoneal macrophages (PMs) were obtained from mice after intraperitoneal thioglycollate injection (22). Murine macrophage RAW264.7 cells were purchased from the American Type Culture Collection (Manassas, VA, USA). RAW264.7 cells and PMs were cultured in DMEM, with 10% fetal bovine serum, 100 μg/ml streptomycin, and 100 U/ml penicillin. Cells were maintained at 37°C in a humidified atmosphere of 5% CO₂ and 95% O₂.

Survival test

C. albicans was cultured on a yeast mold plate at 25°C for 1 d, and a single colony was inoculated into 10 ml yeast malt broth at 30°C for 24 h. C. albicans (1 × 10⁶) was injected into mice peritoneum. The intraperitoneal administration of vehicle or TauCl was performed 3 times a week. Mouse survival (%) was assessed twice daily.

Phagocytosis assay

C. albicans and curdlan were stained with 1 mg/ml FITC in carbonate-bicarbonate buffer (pH 9.6). C. albicans (1 × 10⁶) and curdlan (2.5 mg/mouse) were administered intraperitoneally, followed by injection of vehicle or TauCl (20 mg/kg, i.p.), and mice were euthanized 12 h later. Peritoneal leukocytes were harvested by washing with 3 ml of PBS containing 3 mM EDTA. For phagocytosis in vitro, exudate cells were labeled with allophycocyanin-conjugated anti-F4/80-antibody (macrophage marker). All samples were analyzed with BD FACS Calibur Flow Cytometer (Becton Dickinson, Franklin Lakes, NJ, USA) and FlowJo software (Tree Star, Ashland, OR, USA). Dead cells were excluded by 7-aminoactinomycin D (BioLegend, San Diego, CA, USA) staining.

Flow cytometry analysis

The cells were washed with PBS containing 0.5% bovine serum albumin (BSA). To block nonspecific antibody binding to Fc receptors, the cells were pretreated with unlabeled isotype control antibodies, and then stained with CD16/32 antibody. Fluorescence-conjugated dectin-1 and F4/80 (Thermo Fisher Scientific) were added to samples by incubation on ice for 30 min. After washing the cells with PBS, dectin-1 expression was analyzed by flow cytometry. To confirm the level of PPAR-γ in inner cells, cells were fixed with 2% formaldehyde in PBS for 30 min at room temperature. A permeabilization of cells was preceded with 0.2% Tween-20 in PBS for 15 min at room temperature and blocked with 2% BSA in PBS for 30 min. Anti-PPAR-γ antibodies in PBS containing 2% BSA were applied for 1 h at 4°C. After washing with PBS, cells were incubated with FITC-conjugated secondary antibody for 1 h. Dead cells were excluded by 7-aminoactinomycin D staining. All samples were analyzed by BD FACS Calibur Flow Cytometer and FlowJo software.
RT-PCR

Total RNA was isolated from macrophages using Trizol (Thermo Fisher Scientific) according to the manufacturer’s instruction. To generate cDNA, total RNA was reverse transcribed with murine leukemia virus reverse transcriptase (Promega, Madison, WI, USA). Amplification of genes was performed by PCR analysis using Solg 2× Taq PCR Smart Mix (SolGent, Daejeon, South Korea) according to instruction from the manufacturer. The PCR products were analyzed with 2% agarose gel and stained with SYBR Green for visualization. The mRNA levels were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Western blot analysis

Whole-cell extracts were prepared by suspending the cells in the cell lysis buffer (Cell Signaling Technology, Danvers, MA, USA)
containing protease inhibitors (F. Hoffmann-La Roche, Basel, Switzerland) for 1 h on ice, followed by centrifugation for 15 min at 12,000 g. The protein concentration was determined with BCA protein assay reagents (Thermo Fisher Scientific). The protein samples were solubilized with SDS-PAGE sample-loading buffer and boiled for 5 min. Protein was electrophoresed on SDS-PAGE and transferred to PVDF membranes. The membranes were blocked with 5% fat-free dry milk in PBS containing 0.1% Tween-20 buffer for 1 h at room temperature and incubated with primary antibody, diluted at 1:1000 in 3% fat-free dry milk–PBS containing 0.1% Tween-20, overnight at 4°C. After washing to remove primary antibodies, the blots were incubated with horseradish peroxidase–conjugated secondary

![Image](https://example.com/image1)

**Figure 2.** TauCl augments phagocytosis through up-regulation of dectin-1 expression in PMs. A) The level of dectin-1 in PMs following *C. albicans* infection was detected by flow cytometry. B) The proportion of PMs expressing dectin-1 in the curdlan-induced murine peritonitis model was quantified by flow cytometry. C) The expression of TauCl-induced dectin-1 in the thioglycollate-elicited PMs was analyzed by flow cytometry and RT-PCR. Clec7a, dectin-1 mRNA. (E, F) Protein and mRNA levels of dectin-1 in the harvested primary macrophages treated with vehicle or TauCl (0.5 mM) for 36 h were measured. All data were analyzed by Student’s *t* test. *P < 0.05, **P < 0.01, ***P < 0.001.
antibody. The immunoblot was incubated with the ECL according to the manufacturer’s instruction and visualized with LAS 4000 (FujiFilm, Tokyo, Japan).

Transfection with PPAR-γ small interfering RNA

PPAR-γ small interfering RNA (siRNA; Bioneer, Daejeon, South Korea) was transfected into RAW264.7 cells with lipofectamine RNAi-Max reagents (Thermo Fisher Scientific) according to the manufacturer’s instructions.

Immunohistochemical analysis

PMs from mice treated with curdlan-FITC were fixed with 2% formaldehyde. The cells were washed in PBS and blocked with 3% BSA in Tris-buffered saline and Tween 20. Anti-F4/80 was applied overnight at 4°C, and then, macrophages were washed in PBS. Fluorophore-conjugated secondary antibodies were incubated for another 1 h at room temperature. Samples were imaged at ×20 using an Eclipse Ti-U inverted microscope integrated with NIS-Elements imaging software (Nikon, Tokyo, Japan).

Statistical analysis

All data are presented as means ± se, and statistical analysis was completed in SigmaPlot 12 software (Systat Software, San Jose, CA, USA). Statistical significance was performed using the Student’s t test.

RESULTS

TauCl potentiates phagocytic activity of macrophages

Our previous study demonstrated that TauCl promotes the clearance of apoptotic cells, a process termed efferocytosis, by inducing expression of scavenger receptors on the surface of macrophages during the resolution of inflammation (23). In this study, we investigated the effect of TauCl on the phagocytic function of macrophages to elucidate its role in the host defense against microbial infection. First, we examined whether TauCl administration could protect mice from fungal infection. Injection of a lethal dose of C. albicans to the peritoneum of mice resulted in severe systemic toxicity, leading to a robust reduction in the survival rate (Fig. 1A). The lethal effects of this fungal pathogen were ameliorated by intra-peritoneal administration of TauCl. To investigate the role of TauCl in host defense to fungal infection, we

Figure 3. TauCl-induced HO-1 expression is critical for stimulating phagocytosis by macrophages. A) The proportion of dectin-1-expressing PMs from wild-type and HO-1 KO mice was compared by flow cytometry. B) Mice treated with curdlan-FITC (2.5 mg/mouse) for 6 h were given an intraperitoneal injection of vehicle or the HO-1 inhibitor ZnPP (25 mg/kg) 1 h before the TauCl (20 mg/kg) treatment. The proportion of dectin-1-expressing PMs from mice was determined by flow cytometry. C) Phagocytic activity of macrophages was determined by flow cytometry as described in Materials and Methods. D) The proportion of macrophages ingesting curdlan-FITC* (F4/80dectin-1+) was determined by flow cytometry. Histograms represent quantification of flow cytometry. All data were analyzed by Student’s t test. *P < 0.05, **P < 0.01, ***P < 0.001.
examined the possibility that TauCl could potentiate phagocytic activity of macrophages.

Phagocytosis is a key process used by innate immune cells, particularly macrophages, to eliminate pathogens after recognition by surface receptors (2, 23). To assess the effect of TauCl on phagocytosis by macrophages, we performed an in vivo phagocytosis assay by intraperitoneal injection of C. albicans–FITC. As illustrated in Fig. 1B, injection of TauCl to mice increased the phagocytic activity of macrophages. To further confirm the effects of TauCl on phagocytic activity of macrophages, we evaluated the phagocytosis using a cognate ligand of fungi, curdlan, and a β-1,3-glucan polymer derived from Alcaligenes faecalis (24–27). Because curdlan has a specific affinity for dectin-1, it has been used as a model for pure β-1,3-glucan recognized by macrophages and dendritic cells in studying host immune response to fungal infection (28). Curdlan administration led to infiltration of leukocytes to the mouse peritoneum, which peaked at 6 h (29). We treated the mice with TauCl at 6 h after curdlan administration. The assessment of phagocytic activity of PMs was then performed 12 h after treatment of TauCl. The proportion of PMs labeled with F4/80 and engulfing curdlan–FITC was selectively assessed by flow cytometry. PMs from mice treated with TauCl showed a greater proportion of macrophages carrying out engulfment of curdlan (F4/80+curdlan–FITC+) than those from mice injected with curdlan–FITC alone (Fig. 1C). We also confirmed the TauCl-induced macrophage phagocytic activity with immunofluorescence staining. As illustrated in Fig. 1D, TauCl administration potentiated phagocytic activity of macrophages in curdlan-challenged mice.

**TauCl up-regulates dectin-1 expression in macrophages of mice**

Upon recognition of pathogens by phagocytic receptors, macrophages initiate ingestion of fungal particles. Macrophages then mediate an immune response for microbial clearance through their PRRs. A deficiency in these receptors on the surface of macrophages causes a more severe infection because of an inability to activate phagocytic microbicidal functions (30). Dectin-1 is a representative PRR that recognizes β-1,3-glucans of fungi (18, 19). To determine whether TauCl promotes the phagocytic activity of macrophages through up-regulation of PRR expression, we measured the proportion of PMs expressing dectin-1, which has a decisive role in the macrophage clearance of fungi. We then measured expression of dectin-1 in the PMs of mice upon C. albicans infection. TauCl treatment increased the expression of dectin-1 in macrophages (Fig. 2A). Like C. albicans, administration of

![Figure 4.](image-url) **Figure 4.** TauCl-induced CO production has a role in up-regulation of dectin-1 expression in macrophages. A) Representative flow cytometry data illustrate dectin-1 expression of macrophages cotreated with TauCl (0.5 mM) and Hb (10 μM) as a CO scavenger for 36 h. B) Histograms represent dectin-1 expression in PMs from mice injected with thioglycollate alone or thioglycollate plus CORM-3 (10 mg/kg). All data were analyzed by Student’s t test. *P < 0.05.
curdlan alone caused a modest increase in the proportion of dectin-1–expressing PMs as a host adaptive response to acute inflammation (Fig. 2B). However, the proportion of macrophages expressing dectin-1 was further increased in the curdlan plus TauCl–treated group (Fig. 2B). In general, the amounts of residual PMs are not sufficient for use in biochemical analysis. Thioglycollate broth is a medium that is widely used to enrich macrophages (22). To examine the potent role of chlorination of taurine in up-regulation of dectin-1 expression, mice pretreated with thioglycollate were administrated PBS, taurine, or TauCl. Compared with TauCl, the same dose of its parent molecule, taurine, exerted much weaker effect on dectin-1 expression (Fig. 2C). Consistent with the increased protein level, the expression of the dectin-1 mRNA clec7a was also elevated in the PMs of mice treated with TauCl (Fig. 2D). We also measured the protein and mRNA levels of dectin-1 in the thioglycollate-elicited PMs treated ex vivo with TauCl for 36 h. TauCl treatment increased dectin-1 expression at both translational (Fig. 2E) and transcriptional levels (Fig. 2F). Altogether, these data suggest that TauCl contributes to the phagocytosis of fungi through up-regulation of dectin-1 in macrophages.

**TauCl-induced HO-1 expression is critical for enhanced phagocytic activity of macrophages in murine peritonitis**

Our previous studies have demonstrated that TauCl-induced HO-1 expression in macrophages is essential for their efferocytic activity (23). Considering the prominent role of HO-1 in cellular protection against inflammatory insults (31), we attempted to determine whether the TauCl-induced HO-1 expression (23, 32) could contribute to dectin-1 up-regulation to promote the phagocytic activity of macrophages. We verified the role of HO-1 in TauCl-induced dectin-1 expression by use of HO-1 KO mice. TauCl treatment further increased the dectin-1 expression in macrophages during curdlan-induced peritonitis. The expression of dectin-1 induced by TauCl was abrogated in HO-1 KO mice (Fig. 3A). Consistent with that finding, inhibition of HO activity by ZnPP attenuated TauCl-induced enhancement of dectin-1 expression (Fig. 3B). PMs from HO-1–deficient mice treated with TauCl showed significantly lower phagocytic activity (Fig. 3C) compared with those from the wild-type mice upon TauCl treatment. Likewise, pharmacologic inhibition of HO activity with ZnPP abrogated the TauCl-induced stimulation of the phagocytic activity of macrophages (Fig. 3D). These findings indicate that HO-1 expression contributes substantially to the TauCl-mediated potentiation of the phagocytic activity of macrophages.

**Figure 5. CO increases phagocytosis through dectin-1 expression.** A) Mice were pretreated with CORM-3 (10 mg/kg), 3 times per week before intraperitoneal injection of curdlan–FITC (2.5 mg/kg per mouse). After 18 h, mice were euthanized, and peritoneal exudates were collected. The engulfment of curdlan–FITC by macrophages (F4/80+curdlan-FITC+) was detected by flow cytometry. B) Dot plot represents dectin-1 expression of PMs derived from mice treated with curdlan. All data were analyzed by Student’s t test. *P < 0.05.

**CO potentiates the phagocytic activity of murine macrophages through up-regulation of dectin-1 expression**

CO is a gaseous by-product of the HO-1–catalyzed reaction and has been known to have strong anti-inflammatory activity. First, we attempted to confirm the involvement of CO in TauCl-induced up-regulation of dectin-1 with Hb as a scavenger of CO. Scavenging CO resulted in a marked decrease in the proportion of
dectin-1–expressing macrophages treated with TauCl (Fig. 4A). To further verify the critical role of CO in enhancement of phagocytic activity through dectin-1 in macrophages, we used CORM-3. Mice pretreated with thioglycollate were injected with PBS or CORM-3. The proportion of dectin-1–expressing PMs from mice treated with CORM-3 was further elevated (Fig. 4B). As shown in Fig. 5A, the PMs from mice treated with curdlan–FITC plus CORM-3 exhibited increased phagocytic activity compared with those from mice injected with curdlan–FITC alone. CORM-3 also induced up-regulation of dectin-1 expression in cultured murine PMs (Fig. 5B).

**Figure 6.** TauCl up-regulates dectin-1 expression *via* PPAR-γ in macrophages. A) PPAR-γ expression of PMs from mice treated with curdlan was determined by flow cytometry. B) Mice treated with curdlan–FITC (2.5 mg/mouse) for 6 h were given an intraperitoneal injection of vehicle or GW9662 (3 mg/kg), a PPAR-γ inhibitor, 1 h before TauCl (20 mg/kg) treatment. The proportion of PMs expressing dectin-1 was determined by flow cytometry. C) Phagocytic activity of macrophages was measured by flow cytometry. D) The dectin-1 level of PMs cotreated with TauCl and GW9662 (5 μM) was determined by flow cytometry. E, F) RAW264.7 cells were transfected with scrambled or PPAR-γ siRNA for 24 h, followed by treatment with TauCl for an additional 36 h. The protein (E) and mRNA (F) levels of dectin-1 were measured by flow cytometry and RT-PCR, respectively. All data were analyzed by a Student’s *t* test. *p* < 0.05, **p** < 0.01, ***p*** < 0.001.

**PPAR-γ** is a transcription factor that regulates expression of dectin-1 in macrophages (17, 33). Up-regulation of dectin-1 through activation of PPAR-γ augments antifungal host defense (17). This prompted us to determine whether the TauCl-induced HO-1 expression and subsequent dectin-1 up-regulation could be mediated *via* PPAR-γ in PMs. PMs obtained from mice treated with TauCl showed significantly elevated PPAR-γ expression in curdlan-induced peritonitis (Fig. 6A). To further determine whether TauCl-induced dectin-1 expression was mediated by PPAR-γ, we used a PPAR-γ antagonist GW9662. When the mice were cotreated with GW9662 and curdlan, TauCl failed to induce dectin-1 expression in PMs (Fig. 6B). Likewise, GW9662 injection abrogated the TauCl-induced phagocytic activity of PMs (Fig. 6C). In addition, suppression of PPAR-γ with GW9662 resulted in a blockade of dectin-1 up-regulation in macrophages treated with TauCl (Fig. 6D). When the transcriptional expression of PPAR-γ was silenced in RAW264.7 cells with siRNA, TauCl barely induced expression of dectin-1 at either the transcriptional (Fig. 6E) or translational levels (Fig. 6F). These results suggest that PPAR-γ mediates the up-regulation of TauCl-induced dectin-1 expression in macrophages. To further confirm that the increase of HO-1 by TauCl is responsible for PPAR-γ up-regulation in macrophages, TauCl was intraperitoneally administered with thioglycollate in mice. PMs obtained from TauCl-treated mice showed enhanced expression of PPAR-γ as well as HO-1 (Fig. 7A). Pharmacologic inhibition of HO activity attenuated up-regulation of TauCl-induced PPAR-γ expression in PMs (Fig. 7B). In contrast, an intraperitoneal...
injection of CORM-3 induced expression of PPAR-γ in PMs (Fig. 7C).

DISCUSSION

Humans retain fungal flora as part of their microbiota, which can become pathogenic factors when the systemic immunity of host is changed. Therefore, understanding the mechanisms by which host immunity mediates the resolution of fungal infections is essential (34). Phagocytes, such as macrophages, have a key role in the clearance of pathogens through their PRRs, which detect the microbial PAMPs (6, 35). To eliminate the pathogens efficiently, the macrophages are required to stimulate expression of the phagocytic receptor (2). Upon an acute inflammation response, some endogenous proresolving mediators are produced to facilitate the phagocytic actions of macrophages, which is required for timely resolution of the inflammation (8, 36).

TauCl, a metabolite of taurine produced by activated neutrophils, is released to the site of inflammation. TauCl is a long-lived, mild oxidant that inhibits invasion of pathogens (15). TauCl kills pathogens directly by transferring the chlorine atom to the amino groups in the pathogen membrane (13). The subsequent formation of a chlorine cover, made up of covalent N–Cl bonds, impairs the viability of the pathogens, including bacteria and fungi (37). Several studies suggest that TauCl functions as an endogenous signaling molecule that regulates the generation of pro- and anti-inflammatory mediators in macrophages at the inflamed site (14, 38). We have previously demonstrated that TauCl enhances the efferocytic activity of macrophages (23). Notably, TauCl-induced increases in HO-1 activity are associated with up-regulation of scavenger receptors expressed on the surface of macrophages, which facilitate their recognition and engulfment of apoptotic neutrophils in the inflammatory microenvironment (23).

Phagocytic receptors trigger phagocytosis after recognition of particular molecular patterns expressed the surface of pathogens. The pathogenic fungal β-1,3-glucans is recognized by dectin-1, a PRR expressed on macrophages (2). We found that TauCl stimulates phagocytosis through up-regulation of dectin-1 expression. Phagocytosis presents engulfed antigens efficiently to initiate an adaptive immune response (2). Dectin-1 regulates several Th17-associated cytokines, which influence the differentiation of activated CD4+ T-cells (39, 40). Thus, it seems likely that TauCl-mediated stimulation of phagocytosis could increase antifungal effects through activation of immune responses.

Acute inflammation that occurs upon microbial infection gives rise to transient induction of HO-1, which possesses an anti-inflammatory capability. The enhanced HO-1 expression protects the host tissue from injuries caused by pathogens through multiple mechanisms (41). Consistent with that, HO-1 knock down increases susceptibility to microbial infection, which is associated with

![Figure 7. HO-1 induction by TauCl is required for dectin-1 up-regulation by PPAR-γ in macrophages.](image-url)

A) Mice were treated with thioglycollate and TauCl as described in Materials and Methods. The protein expression levels of PPAR-γ and HO-1 in macrophages were assessed by Western blot analysis. B) To determine whether HO-1 activation is required for PPAR-γ induction by TauCl, mice were cotreated with TauCl (20 mg/kg) and ZnPP (25 mg/kg). After 3 d, mice were euthanized to collect PMs. The PPAR-γ protein level of macrophages was measured by Western blot analysis. C) To verify that CO, as a by-product of the HO-1 reaction, is required for PPAR-γ expression in macrophages, mice were co-injected with thioglycollate and CORM-3. The protein level of PPAR-γ was measured by Western blot analysis. All data were analyzed by Student’s t test. **p < 0.01, ***p < 0.001.
an inability of the macrophages to protect the host from pathogens (42). TauCl has been shown to exert cytoprotective and anti-inflammatory effects through HO-1 up-regulation (14, 43). This prompted us to speculate that HO-1 up-regulation in macrophages might be critical for its antifungal activity. In spite of the several studies demonstrating that elevated HO-1 expression enhances bacterial clearance (44, 45), little is known about the molecular mechanisms by which HO-1 induction stimulates phagocytosis. In the present study, we found that TauCl-induced HO-1 overexpression facilitates phagocytosis through dectin-1 up-regulation.

CO generated because of HO-1 induction is an important molecule that acts as an anti-inflammatory substance and a proresolving mediator (8, 46). Although CO is a relatively inert gas in biologic systems, it can maintain cellular protection against inflammatory insults (47). Our present study indicates that HO-1–derived CO production by TauCl can up-regulate dectin-1 expression and thereby stimulate fungal clearance by macrophages. Recent studies suggest that fungal-antigen recognition by dectin-1 leads to LC3-associated phagocytosis (LAP) (48). Initiation of LAP accelerates internalization of microbial particles and phagosome maturation for degradation of the phagosome contents (49). HO-1 induction and subsequent CO production are potentially involved in autophagy for enhancement of host protection (50, 51). It will be worthwhile to determine whether TauCl-induced CO production is involved in LAP acceleration by dectin-1 during fungal clearance.

PPAR-γ has an essential role in host response to inflammation by regulating expression of macrophage phagocytic receptors, such as dectin-1 (17, 52). PPAR-γ is also required for the alternative activation of macrophages, which mediate anti-inflammatory effects (52). Activation of PPAR-γ promotes uptake and killing of C. albicans by macrophages (17, 53). The up-regulation of dectin-1 by PPAR-γ ligands in macrophages promotes phagocytosis and triggers resolution of candidiasis (17, 33, 54, 55). Moreover, dectin-1 activation by a particulate yeast-derived β-glucan resulted in conversion of polarized immunosuppressive M2 macrophages into an M1-like phenotype with potent immune-stimulating activity (56). In this study, we found that TauCl-induced HO-1 expression up-regulates dectin-1 through PPAR-γ in macrophages. Several studies have suggested that HO-1–derived CO enhances PPAR-γ activity, thereby regulating inflammatory processes (44, 57). CO facilitates the termination of acute inflammation by stimulating production of proresolving lipid mediators (58). PPAR-γ is a member of the nuclear receptors of lipid ligand-inducible transcription factors (59). We speculate that CO produced by TauCl-induced HO-1 up-regulation may trigger activation of PPAR-γ. Further studies will be necessary to determine whether TauCl-induced CO production potentiates the phagocytic activity of macrophages through PPAR-γ activation.

In summary, TauCl released from apoptotic neutrophils at inflamed sites enhances phagocytic activity of macrophages by stimulating expression of HO-1 and subsequently dectin-1 (Fig. 8). Specifically, CO, a byproduct of the HO-1–catalyzed reaction, has a critical role as a putative signaling molecule in mediating TauCl-induced phagocytosis. Our study suggests that TauCl, a metabolite of taurine produced in the inflammatory microenvironment, has a powerful therapeutic potential to act as a modulator of phagocytic activity in the inflammatory disorders.

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**AUTHOR CONTRIBUTIONS**

S. H. Kim conducted most of the research and wrote the paper; X. Zhong and W. Kim helped with the research; K. Kim and Y. Joe contributed experimental materials and techniques; Y.-G. Suh, C. Kim, H. T. Chung, and Y.-N. Cha...
analyzed the data and participated in discussions; and Y.-J. Surh guided the entire study and edited the manuscript.

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