The hydrogen sulfide signaling in macrophages: A foe or friend?

Xin-Qiang Wang[1], Wang Congyi[2], Fei Sun[2], Jia-hui Luo[2], Tian-tian Yue[2], Fa-xi Wang[2], Chun-liang Yang[2], and Shu Zhang[2]

[1]Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology
[2]The Center for Biomedical Research, Tongji Hospital

April 28, 2020

Abstract

Hydrogen sulfide (H2S) is the latest identified small gaseous mediator featured by its lipophilic nature to freely permeate the biological membranes. Initially, H2S was recognized by its roles in neuronal activity and vascular relaxation, which makes it an important molecule involved in paracrine signaling pathways. Recently, the immune regulatory function of gasotransmitters, H2S in particular, is increasingly being appreciated. Endogenous H2S level has been linked to macrophage activation, polarization, and inflammasome formation. Mechanistically, H2S-induced protein S-sulfhydration suppresses several inflammatory pathways including NF-ϰB and JNK signaling. Moreover, H2S serves as a potent cellular redox regulator to modulate epigenetic alterations and to promote mitochondrial biogenesis in macrophages. Here in this review, we intend to summarize the recent advancements of H2S studies in macrophages, and to discuss with focus on the therapeutic potential of H2S donors by targeting macrophages. The feasibility of H2S signaling component as a macrophage biomarker under disease conditions would be also discussed.

The hydrogen sulfide signaling in macrophages: A foe or friend?

Fei Sun[1,*], Jia-Hui Luo[1,*], Tian-Tian Yue[1], Fa-Xi Wang[1], Chun-Liang Yang[1], Shu Zhang[1], Xin-Qiang Wang[2,#], Cong-Yi Wang[1,#]

[1]The Center for Biomedical Research, Department of Respiratory and Critical Care Medicine, Key Laboratory of Pulmonary Diseases of Health Ministry, Tongji Hospital, Tongji Medical College, Huazhong University of Sciences and Technology, Wuhan, China.

[2]Institute of Organ Transplantation, Key Laboratory of Organ Transplantation, Ministry of Education, NHC Key Laboratory of Organ Transplantation, Key Laboratory of Organ Transplantation, Chinese Academy of Medical Sciences, Tongji Hospital, Tongji Medical College, Huazhong University of Sciences and Technology, Wuhan, China.

*These authors contributed equally to this work.

#Correspondence to Xin-Qiang Wang (email:*13098892232@163.com*) and Cong-Yi Wang(email:*wangcy@tjh.tjmu.edu.cn*).

Summary

Hydrogen sulfide (H_2S) is the latest identified small gaseous mediator featured by its lipophilic nature to freely permeate the biological membranes. Initially, H_2S was recognized by its roles in neuronal activity and vascular relaxation, which makes it an important molecule involved in paracrine signaling pathways. Recently, the immune regulatory function of gasotransmitters, H_2S in particular, is increasingly being appreciated. Endogenous H_2S level has been linked to macrophage activation, polarization, and inflammasome formation. Mechanistically, H_2S-induced protein S-sulfhydration suppresses several inflammatory pathways including NF-ϰB and JNK signaling. Moreover, H_2S serves as a potent cellular redox regulator to modulate

epigenetic alterations and to promote mitochondrial biogenesis in macrophages. Here in this review, we intend to summarize the recent advancements of H_2S studies in macrophages, and to discuss with focus on the therapeutic potential of H_2S donors by targeting macrophages. The feasibility of H_2S signaling component as a macrophage biomarker under disease conditions would be also discussed.

Key words:

H_2S, macrophage function, S-sulfhydration, redox regulation, epigenetics

Introduction

Gasotransmitters are a group of ubiquitous small gaseous signaling molecules, which mainly consist of nitric oxide (NO), carbon monoxide (CO) and hydrogen sulfide (H_2S) (1). Their lipophilic nature allows them to freely permeate through the biological membranes and to play an essential role in the regulation of cellular processes (1, 2). Indeed, dysregulation of gasotransmitter system is associated with numerous diseases ranging from neurological disorders to musculoskeletal abnormalities (3-5). Recently, encouraging results have further indicated a regulatory role for gasotransmitters in immune cells (2). In particular, macrophage, as the patrolling sentinel in the immune system, is extensively regulated by these gaseous mediators (6).

Upon activation, the classically activated (M1) macrophages up-regulate the expression of inducible nitric oxide synthase (iNOS), and catalyze the transformation of L-arginine to NO. Elevated NO along with the production of reactive nitrogen species (RNS) is indispensable for the optimal anti-microbial activity and the secretion of inflammatory cytokines such as IL-6, TNFα and Interferons (7, 8). On the other hand, the alternatively activated (M2)macrophages highly express the hallmark enzyme Arginase1 (Arg1), which out-competes the activity of iNOS on L-arginine availability and reduces the NO production (9). Therefore, the fluctuation of NO metabolism serves as a key molecular switch for control of macrophage function to dynamically regulate the initiation or resolution of an inflammatory response. In contrast to NO, CO, a heme metabolism product produced by the heme oxygenase 1-3 (HO 1-3), attenuates macrophage activation and therefore, HO1 over-expression in myeloid lineages favors M2 program in macrophages and implies better outcome in liver transplant patients (10). Consistently, HO-1 deficiency leads to increased M1 macrophages along with enhanced inflammatory infiltration following ischemia-reperfusion injury (10). Similarly, CO suppresses lipopolysaccharide (LPS) induced macrophage activation and induces the secretion of IL-10, which involves its effect on the activation of mitogen-activated protein kinase kinase 3 (MKK3) (11).

H_2S, the latest identified gasotransmitter, was first recognized as a smelly and environmental toxic gas (12). Past two decades of studies revealed that H_2S can be generated endogenously and work as an autocrine signaling molecule (3, 13, 14). In mammals, three enzymes including cystathionine γ-lyase (CSE), cystathionine β-synthase (CBS), and the 3-mercaptopyruvate sulfur transferase (3-MST), are responsible for H_2S generation (15, 16). Specifically, CSE and CBS catalyze de-sulfhydration of cysteine to generate H_2S, while MST induces H_2S production by regulating the enzymatic activity of cysteine aminotransferase (CAT) (17, 18). The essential role of H_2S signaling in T cell biology has been well addressed, in which ablation of CBS and CSE leads to impaired T cell activation and proliferation (19). Mice deficient in *CBS* also manifest reduced regulatory T cells (Tregs) along with massive inflammatory infiltration, which could be reversed by H_2S donor supplementation (20).

Interestingly, unlike its effect on T cells, the regulatory role of H_2S signaling in macrophages, however, is much more complex. It seems that H_2S actively impact macrophage on its activation, polarization and inflammasome formation through distinct mechanistic pathways. Particularly, macrophages likely also set the threshold for the activation of H_2S signaling under various stimuli. Herein, we aim to summarize the regulatory mechanisms underlying H_2S signaling, and discuss with focus for the impact of H_2S signaling on the regulation of macrophage functionality.

1. The Regulatory Mechanisms Underlying H_2S Signaling

H_2S signaling plays a critical regulatory role in diverse immune responses, which involves H_2S-induced protein S-sulfhydration, cellular redox homeostasis and epigenetic chromatin remodeling (**Figure1**). In this section,

we briefly summarize the above regulatory mechanisms underlying H_2S signaling.

1.1 Protein S-sulfhydration

H_2S induced protein S-sulfhydration is a novel post-translational modification (PTM) occurring on specific cysteine (Cys) residues of target proteins, by which it regulates the biological activity of targeted proteins. S-sulfhydration of key enzymes, receptors and transcriptional factors contribute a major part to H_2S signaling and its regulatory function. Kir6.1, a subunit of ATP sensitive potassium channels (K_{ATP}), is S-sulfhydrated at Cys43, which promotes K_{ATP} channel activity and improves vasodilatation (21). Other ion channels such as voltage-activated calcium channels, and transient receptor potential (TRP) channel protein TRPV6 and TRPV4, were also suggested to be S-sulfhydrated, thereby regulating calcium flux (22, 23). Together, these events perfectly explain the effect of endogenous H_2S and exogenous H_2S donors on vascular relaxation.

Metabolic reprogramming and stress responses including oxidative stress and endoplasmic reticulum (ER) stress are critical regulators in immune cells and their fate decision. Other than the well-known role in cardiovascular system, H_2S-mediated protein S-sulfhydration also engages in the metabolic processes and cellular stress responses. S-sulfhydration of peroxisome proliferator activated receptor-γ (PPARγ) at Cys139 enhances its DNA binding activity and the subsequent expression of adipogenic genes, thus increasing glucose uptake and lipid metabolism (24). Additionally, H_2S promotes the activities of PPARγ coactivator related protein (PPRC), alpha subunit of ATP synthase (ATP5A1) and interferon regulatory factor1 (IRF1) via S-sulfhydration, by which it stimulates mitochondrial biogenesis and protects from mitochondrial dysfunction (25-27). P66Shc is an upstream activator of mitochondrial redox signaling, and studies suggested that H_2S protects neuronal cells against stress-induced senescence by inducing its S-sulfhydration at Cys59 residue (28). H_2S also induces Keap1 S-sulfhydration (Cys151, Cys226 and Cys613) to promote the dissociation of Keap1-Nrf2 complex, thereby releasing Nrf2 to transcribe the expression of antioxidant genes (29, 30). Similarly, PTP-1B is a protein tyrosine phosphastase related to the deactivation of protein kinase RNA-like ER kinase (PERK), while H_2S mediates PTP-1B S-sulfhydration at Cys215 to inhibit its enzymatic activity, thereby activating PERK pathway to alleviate ER stress (31).

It is worthy of note that some immune regulatory molecules are the direct targets for H_2S induced S-sulfhydration. For example, S-sulfhydration of nuclear transcription factor Y subunit beta (NFYB) at Cys105 increases the transcription of the ten-eleven translocation (Tet) genes (32). Tet1 and Tet2 in turn bind to the regulatory regions within the *Foxp3* gene to maintain the hypomethylation status of its promoter and the conserved non-coding sequence2 (CNS) region, thereby ensuring Foxp3 expression and the stability of Treg cell lineage (20). Similarly, S-sulfhydration of the free thiol group Cys38 in p65 inhibits NF-ϰB activity in macrophages (33). Moreover, S-sulfhydration of c-Jun at Cys269 attenuates hydrogen peroxide (H_2O_2)-induced NLRP3 inflammasome activation and reduces IL-1β production in macrophages (34).

1.2 Cellular redox homeostasis

Theoretically, most H_2S can dissolve in surface water and dissociate into HS^- under normal circumstances (37,PH=7.4) (35), and HS^- in turn could serve as a powerful one-electron chemical reductant to scavenge ROS. In reality, however, the physiological concentration of H_2S is at the sub-micromolar level (36), which is too low for H_2S to act as a direct antioxidant. Nonetheless, although H_2S itself is recognized not high enough to act as a reducing agent, it can, in fact, exert potent antioxidant effects in alternative manners. Specifically, other than the aforementioned Keap1 S-sulfhydration mediated pathway, hypoxia inducible factor 1α (HIF-1α) also serves as another important molecule downstream of H_2S signaling (37). Studies in THP-1 cells, a human macrophage cell line, revealed that H_2S induces HIF-1a nuclear translocation to enhance the expression of glucose transporter GLUT1 (37). It was also found that H_2S could activate the antioxidant Nrf2/HO-1 pathway by stimulating the p38 mitogen-activated protein kinase (MAPK) activity (37). Therefore, H_2S has been found to attenuate LPS-induced acute lung injury by reducing oxidative and nitrative species (38), and H_2S administration improves glutathione (GSH) level along with alleviated lipid peroxidation and allergic lung inflammation (39). Collectively, as a negative regulator in cellular redox homeostasis, H_2S exhibits anti-inflammatory potency amid stress related inflammatory disorders.

1.3 Epigenetic chromatin remodeling

Another critical mechanism underlying H_2S signaling is that H_2S also manifests a remarkable capacity to regulate epigenetic chromatin remodeling. Apart from the above introduced NFYB-Tet pathway, which mediates DNA demethylation of the*Foxp3* regulatory regions in Treg cells, H_2S exhibits high potency to remodel chromatin structure through regulation of histone modifications in macrophages.

The Jumonji domain-containing protein 3 (JMJD3) is a histone 3 Lys27 (H3K27) demethylase and plays a critical role in chromatin remodeling (40). There is evidence that LPS upregulates CSE expression in macrophages in a mouse model with septic shock, and enhanced CSE in turn inhibits JMJD3 expression to increase H3K27me3 levels, thereby attenuating LPS-mediated inflammatory response (41). Studies in macrophages further noted that H_2S is capable of suppressing histone acetylation at the IL-6 and TNF-α promoter, by which it inhibits chromatin openness to repress the transcription of inflammatory cytokines following LPS stimulation (42). These results suggest that the CSE/H_2S signaling could be vital to prevent uncontrolled inflammatory responses.

Heretofore, the major mechanism underlying H_2S signaling is likely attributed to the S-sulfhydration of substrate proteins. Moreover, the impact of H_2S signaling on the regulation of redox homeostasis and chromatin remodeling seems independent of S-sulfhydration (**Figure 1**), but additional studies would be necessary to fully address this issue. It should be also important to keep in mind that characterization of additional unidentified S-sulfhydration proteins would help to completely clarify the regulatory mechanisms.

2. H_2S signaling in maintaining the M1/M2 homeostasis in macrophages

As described earlier, macrophages display different functional phenotypes depending on their residing environmental milieu. For simplicity, they are classified into two distinct subtypes: one is classically activated (M1) macrophages, and the other is alternatively activated (M2) macrophages. LPS and IFN-γ induce the generation of M1 macrophages, which then augment the production of pro-inflammatory cytokines. In contrast, M2 macrophages are elicited by glucocorticoids or type II cytokines such as IL-4, IL-13, and IL-10. M2 macrophages are responsible for wound healing, tissue repair and the resolution of inflammation, thus generally regarded as an anti-inflammatory cell type.

Recent studies provided compelling evidence that H_2S signaling is implicated in dictating macrophage polarizations. Initially, the endogenous hydrogen sulfide was found to attenuate LPS-induced oxidative stress and inflammatory damage by inhibiting Nox4-ROS signaling pathway in macrophages (43). GYY4137, a novel hydrogen sulfide-releasing molecule, was confirmed to inhibit rat endotoxic shock and mucosal wound through abrogating M1 program in macrophages (44, 45). Similarly, FW1256, another slow-releasing hydrogen sulfide donor, was further noted to exhibit anti-inflammatory properties by reducing the production of inflammatory mediators such as TNF-α, IL-6, PGE2,IL-1β, COX-2 and NO in macrophages (46). Subsequent mechanistic studies demonstrated that NaHS promotes macrophage M2 polarization by enhancing mitochondrial biogenesis and fatty acid oxidation (47). Similar results were also observed in the central nerve system (CNS), in which H_2S exerts neuroprotection against hypoxia-induced neurotoxicity through induction of M2 program in microglia cells by inhibiting iNOS, NF-κB, ERK and p38 MAPK signaling pathways (48). Therefore, H_2S signaling serves as a critical regulatory mechanism to maintain the homeostatic M1/M2 balance in the setting of inflammatory resolution.

3. H_2S signaling in macrophage activation and inflammasome formation

It was noted that LPS-stimulated macrophages and adipose tissue macrophages (ATMs) derived from diet-induced obese (DIO) mice manifest lower intracellular concentration of H_2S (49), suggesting that depletion of macrophage H_2S content both occurs during acute (LPS-induced) and chronic (obesity) inflammatory conditions. Indeed, oxidized low-density lipoprotein (oxLDL) induces the CSE promoter to undergo DNA hypermethylation in macrophages, leading to attenuated CSE transcription and H_2S production in favor of inflammatory responses (50), which involves the activation of JNK/NF-κB signaling (51). Similarly, homocysteine (HCy) induces DNA hypermethylation in the CSE promoter in macrophages, through which

it exaggerates inflammation by inhibiting CSE-H_2S signaling (52) (**Figure 2**).

In line with above observations, a time-dependent change of H_2S content in macrophages was found following activation. A decrease of H_2S level in murine macrophages following 24 h of LPS or IFN-γ stimulation was observed, but the H_2S content was restored to normal level after 48 h of stimulation, which was associated with the feedback regulation between CBS and CSE (53). It is worthy of note that H_2S production was in paralleled with LPS-induced macrophage late stage apoptosis, which could be blocked by the addition of H_2S inhibitor (54). Therefore, it is possible that sustained LPS stimulation renders macrophages to undergo apoptosis through the production of H_2S (**Figure 2**).

Macrophages not only sense exogenous pathogen associated molecular patterns (e.g., LPS) derived from microorganisms, but also respond to endogenous stimuli. The most commonly seen endogenous insults originate from harmful metabolites, such as excessive free fatty acids (FFAs) and oxLDL. Interestingly, these metabolites alone could lead to abnormal macrophage activation, while they could also serve as the second signals essential for inflammasome formation. Inflammasome is a complex of proteins found in macrophages that regulates the activation of caspase enzymes and induces the secretion of pro-inflammatory cytokines (e.g. IL-1β and IL-18). Importantly, recent studies demonstrated that both exogenous and endogenous H_2S inhibit NLRP3 inflammasome activation and reduce inflammatory cytokine production in macrophages (55). In particular, upregulation of H_2S content by treating the cells with NaHS reduces the expression level of inflammasome associated proteins such as TXNIP, NLRP3, ASC and Caspase-1 by inhibiting thioredoxin interacting protein-NLRP3 (TXNIP-NLRP3) signaling pathway (56). Taken together, H_2S signaling not only directly represses macrophage activation, but also inhibits inflammasome formation, thereby attenuating inflammatory responses.

4. The therapeutic potential for targeting H_2S signaling in macrophages

Macrophages are critical participants in the immune system, which are involved in innate immunity and also help to recruit other immune cells for adaptive immune responses. Macrophages can be found essentially in all tissues and their dysfunction is linked to a variety of diseases. Dysregulation of macrophages is related to various diseases ranging from infection to metabolic disorders, wherein H_2S donors exhibit significant therapeutic potential.

It has been well recognized that enhanced H_2S signaling in macrophages abrogates the progression of septic shock (44), a severe inflammatory disorder caused by bacterial infection and now faces up with limited therapeutics in clinic. Microglia, a specialized macrophage in the nerve system, is involved in the pathogenesis of Alzheimer's and Parkinson's disease. Given the role of H_2S signaling in the resolution of neuronal inflammation (57-59), H_2S donors are proven to be effective in numerous neuronal disorders (48, 59). Similarly, since H_2S reduces FFAs and oxLDL induced metabolic stress and inflammasome formation, H_2S donors could inhibit foam cell formation and attenuate the release of pro-inflammatory cytokines, thus leading to the amelioration of arterial atherosclerosis and other inflammasome associated diseases such as DSS-induced colitis (56, 60, 61). As aforementioned, intracellular concentration of H_2S was lower in ATMs of obese mice, and not surprisingly, exogenous supplementation of H_2S donors could curb the development of obesity and the subsequent metabolic syndromes (49). Together, these results support that targeting H_2S signaling in macrophages could be a viable approach against immune and metabolic disorders in clinical setting.

Concluding remarks and perspectives

It would be important to note that the relationship between H_2S signaling and macrophage functionality is reciprocal and dynamic. H_2S actively modulates macrophage activation, polarization and inflammasome formation (**Figure1**), and macrophages in turn influence the intrinsic H_2S synthetic machinery following external stimuli. Specifically, upon LPS stimulation, H_2S production is shut down at the early stage to facilitate pro-inflammatory cytokine secretion, while at the late stage, the H_2S content becomes increased for induction of those mission-completed macrophages to undergo apoptosis. This complex feed-back loop underpins the multifaceted function of macrophages, which reflects a fine control of macrophage-mediated immune response (**Figure 2**).

Generally, H_2S induces S-sulfhydration of key signaling molecules, such as p65 and c-Jun, to impact on NF-κB pathway and canonical NLRP3 inflammasome formation, while H_2S also regulates cellular redox homeostasis and chromatin remodeling to affect macrophage function. However, we cannot exclude the possibility that additional unrecognized S-sulfhydrated proteins could be also engaged inH_2S signaling. As for the regulation of cellular redox homeostasis, it is intriguing that H_2S induced S-sulfhydration shares great similarity with GSH mediated S-glutathionylation (62, 63), and both of which even possess the same substrate, PTP1B (31, 64). There is evidence that S-glutathionylation regulates redox homeostasis (65), and a typical example is MKP1, which has been verified to be a substrate for S-glutathionylation (66). It is therefore plausible that H_2S could either directly mediates MKP1 S-sulfhydration to regulate macrophage redox homeostasis, or indirectly influences MKP1 S-glutathionylation by elevating GSH levels, which could perfectly explain the inhibitory effect of H_2S on MAPK signaling.

Macrophages demand distinct intracellular metabolic pathways depending on their functional state. The activation of M1 macrophages by LPS or IFN-γ is associated with higher glycolysis along with attenuated tri-carboxylic acid (TCA) cycle and mitochondrial oxidative phosphorylation (OXPHOS) (67). In contrast, M2 macrophages require higher mitochondrial biogenesis, fatty acid uptake and fatty-acid oxidation (FAO) (68, 69). Collectively, those discoveries support that H_2S mediated metabolic reprogramming finely control the initiation and resolution of an inflammatory response (47). Therefore, a better understanding of the role for H2S signaling in macrophages would demonstrate great potential to develop therapies against either acute or chronic inflammatory responses in clinical settings of patients with immune or metabolic disorders.

Acknowledgment

FS and JHL wrote the paper; TTY, FXW and CLY revised the manuscript; SZ gave us pivotal suggestions; XQW and CYW conceptualized and revised the manuscript. Our work was supported by the Ministry of Science and Technology (2016YFC1305002 and 2017YFC1309603), the National Natural Science Foundation of China (81530024, 91749207, 81920108009, and 81770823), the NHC Drug Discovery Program (2017ZX09304022-07), the Integrated Innovative Team for Major Human Disease Programs of Tongji Medical College, Huazhong University of Science and Technology, and the Innovative Funding for Translational Research from Tongji Hospital.

Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

1. Wang R. Gasotransmitters: growing pains and joys. Trends in Biochemical Sciences. 2014;39(5):227-32.

2. Fagone P, Mazzon E, Bramanti P, Bendtzen K, Nicoletti F. Gasotransmitters and the immune system: Mode of action and novel therapeutic targets. European Journal of Pharmacology. 2018;834:92-102.

3. Paul BD, Snyder SH. Gasotransmitter hydrogen sulfide signaling in neuronal health and disease. Biochemical Pharmacology. 2018;149:101-9.

4. Zhang L, Wang Y, Li Y, Li L, Xu S, Feng X, et al. Hydrogen Sulfide (H2S)-Releasing Compounds: Therapeutic Potential in Cardiovascular Diseases. Frontiers in Pharmacology. 2018;9:1066.

5. Wallace JL, Ferraz JGP, Muscara MN. Hydrogen sulfide: an endogenous mediator of resolution of inflammation and injury. Antioxidants & redox signaling. 2012;17(1):58-67.

6. Lavin Y, Mortha A, Rahman A, Merad M. Regulation of macrophage development and function in peripheral tissues. Nature reviews Immunology. 2015;15(12):731-44.

7. Sica A, Mantovani A. Macrophage plasticity and polarization: in vivo veritas. The Journal of Clinical Investigation. 2012;122(3):787-95.

8. Gautier EL, Yvan-Charvet L. Understanding macrophage diversity at the ontogenic and transcriptomic levels. Immunological Reviews. 2014;262(1):85-95.

9. Gordon S, Martinez FO. Alternative Activation of Macrophages: Mechanism and Functions. Immunity. 2010;32(5):593-604.

10. Zhang M, Nakamura K, Kageyama S, Lawal AO, Gong KW, Bhetraratana M, et al. Myeloid HO-1 modulates macrophage polarization and protects against ischemia-reperfusion injury. JCI Insight. 2018;3(19).

11. Otterbein LE, Bach FH, Alam J, Soares M, Tao Lu H, Wysk M, et al. Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway. Nature Medicine. 2000;6(4):422-8.

12. Wang R. Physiological Implications of Hydrogen Sulfide: A Whiff Exploration That Blossomed. Physiological Reviews. 2012;92(2):791-896.

13. Abe K, Kimura H. The possible role of hydrogen sulfide as an endogenous neuromodulator. The Journal of Neuroscience. 1996;16(3):1066.

14. Zhao W, Zhang J, Lu Y, Wang R. The vasorelaxant effect of H(2)S as a novel endogenous gaseous K(ATP) channel opener. The EMBO journal. 2001;20(21):6008-16.

15. Kabil O, Banerjee R. Redox Biochemistry of Hydrogen Sulfide. Journal of Biological Chemistry. 2010;285(29):21903-7.

16. Powell CR, Dillon KM, Matson JB. A review of hydrogen sulfide (H2S) donors: Chemistry and potential therapeutic applications. Biochemical Pharmacology. 2018;149:110-23.

17. Szabó C. Hydrogen sulphide and its therapeutic potential. Nature Reviews Drug Discovery. 2007;6:917.

18. Li L, Rose P, Moore PK. Hydrogen Sulfide and Cell Signaling. Annual Review of Pharmacology and Toxicology. 2011;51(1):169-87.

19. Miller TW, Wang EA, Gould S, Stein EV, Kaur S, Lim L, et al. Hydrogen Sulfide Is an Endogenous Potentiator of T Cell Activation. Journal of Biological Chemistry. 2012;287(6):4211-21.

20. Yang R, Qu C, Zhou Y, Konkel JE, Shi S, Liu Y, et al. Hydrogen Sulfide Promotes Tet1- and Tet2-Mediated Foxp3 Demethylation to Drive Regulatory T Cell Differentiation and Maintain Immune Homeostasis. Immunity. 2015;43(2):251-63.

21. Mustafa Asif K, Sikka G, Gazi Sadia K, Steppan J, Jung Sung M, Bhunia Anil K, et al. Hydrogen Sulfide as Endothelium-Derived Hyperpolarizing Factor Sulfhydrates Potassium Channels. Circulation Research. 2011;109(11):1259-68.

22. Liu Y, Yang R, Liu X, Zhou Y, Qu C, Kikuiri T, et al. Hydrogen sulfide maintains mesenchymal stem cell function and bone homeostasis via regulation of Ca(2+) channel sulfhydration. Cell stem cell. 2014;15(1):66-78.

23. Naik JS, Osmond JM, Walker BR, Kanagy NL. Hydrogen sulfide-induced vasodilation mediated by endothelial TRPV4 channels. American Journal of Physiology-Heart and Circulatory Physiology. 2016;311(6):H1437-H44.

24. Cai J, Shi X, Wang H, Fan J, Feng Y, Lin X, et al. Cystathionine γ lyase–hydrogen sulfide increases peroxisome proliferator-activated receptor γ activity by sulfhydration at C139 site thereby promoting glucose uptake and lipid storage in adipocytes. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids. 2016;1861(5):419-29.

25. Untereiner AA, Fu M, Módis K, Wang R, Ju Y, Wu L. Stimulatory effect of CSE-generated H2S on hepatic mitochondrial biogenesis and the underlying mechanisms. Nitric Oxide. 2016;58:67-76.

26. Módis K, Ju Y, Ahmad A, Untereiner AA, Altaany Z, Wu L, et al. S-Sulfhydration of ATP synthase by hydrogen sulfide stimulates mitochondrial bioenergetics. Pharmacological Research. 2016;113:116-24.

27. Li S, Yang G. Hydrogen Sulfide Maintains Mitochondrial DNA Replication via Demethylation of TFAM. Antioxidants & Redox Signaling. 2015;23(7):630-42.

28. Xie Z-Z, Shi M-M, Xie L, Wu Z-Y, Li G, Hua F, et al. Sulfhydration of p66Shc at Cysteine59 Mediates the Antioxidant Effect of Hydrogen Sulfide. Antioxidants & Redox Signaling. 2014;21(18):2531-42.

29. Yang G, Zhao K, Ju Y, Mani S, Cao Q, Puukila S, et al. Hydrogen Sulfide Protects Against Cellular Senescence via S-Sulfhydration of Keap1 and Activation of Nrf2. Antioxidants & Redox Signaling. 2012;18(15):1906-19.

30. Hourihan JM, Kenna JG, Hayes JD. The Gasotransmitter Hydrogen Sulfide Induces Nrf2-Target Genes by Inactivating the Keap1 Ubiquitin Ligase Substrate Adaptor Through Formation of a Disulfide Bond Between Cys-226 and Cys-613. Antioxidants & Redox Signaling. 2012;19(5):465-81.

31. Krishnan N, Fu C, Pappin DJ, Tonks NK. H2S-Induced sulfhydration of the phosphatase PTP1B and its role in the endoplasmic reticulum stress response. Science signaling. 2011;4(203):ra86-ra.

32. Oh Soyoung A, Li Ming O. TETs Link Hydrogen Sulfide to Immune Tolerance. Immunity. 2015;43(2):211-3.

33. Du J, Huang Y, Yan H, Zhang Q, Zhao M, Zhu M, et al. Hydrogen Sulfide Suppresses Oxidized Low-density Lipoprotein (Ox-LDL)-stimulated Monocyte Chemoattractant Protein 1 generation from Macrophages via the Nuclear Factor ϰB (NF-ϰB) Pathway. Journal of Biological Chemistry. 2014;289(14):9741-53.

34. Lin Z, Altaf N, Li C, Chen M, Pan L, Wang D, et al. Hydrogen sulfide attenuates oxidative stress-induced NLRP3 inflammasome activation via S-sulfhydrating c-Jun at Cys269 in macrophages. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease. 2018;1864(9, Part B):2890-900.

35. Mathai JC, Missner A, Kügler P, Saparov SM, Zeidel ML, Lee JK, et al. No facilitator required for membrane transport of hydrogen sulfide. Proceedings of the National Academy of Sciences. 2009;106(39):16633.

36. Furne J, Saeed A, Levitt MD. Whole tissue hydrogen sulfide concentrations are orders of magnitude lower than presently accepted values. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2008;295(5):R1479-R85.

37. Lohninger L, Tomasova L, Praschberger M, Hintersteininger M, Erker T, Gmeiner BMK, et al. Hydrogen sulphide induces HIF-1α and Nrf2 in THP-1 macrophages. Biochimie. 2015;112:187-95.

38. Zhang HX, Liu SJ, Tang XL, Duan GL, Ni X, Zhu XY, et al. H₂S Attenuates LPS-Induced Acute Lung Injury by Reducing Oxidative/Nitrative Stress and Inflammation. Cellular Physiology and Biochemistry. 2016;40(6):1603-12.

39. Campos D, Ravagnani FG, Gurgueira SA, Vercesi AE, Teixeira SA, Costa SKP, et al. Increased glutathione levels contribute to the beneficial effects of hydrogen sulfide and inducible nitric oxide inhibition in allergic lung inflammation. International Immunopharmacology. 2016;39:57-62.

40. De Santa F, Totaro MG, Prosperini E, Notarbartolo S, Testa G, Natoli G. The Histone H3 Lysine-27 Demethylase Jmjd3 Links Inflammation to Inhibition of Polycomb-Mediated Gene Silencing. Cell. 2007;130(6):1083-94.

41. Liu S, Wang X, Pan L, Wu W, Yang D, Qin M, et al. Endogenous hydrogen sulfide regulates histone demethylase JMJD3-mediated inflammatory response in LPS-stimulated macrophages and in a mouse model of LPS-induced septic shock. Biochemical Pharmacology. 2018;149:153-62.

42. Rios ECS, Szczesny B, Soriano FG, Olah G, Szabo C. Hydrogen sulfide attenuates cytokine production through the modulation of chromatin remodeling. International journal of molecular medicine.

2015;35(6):1741-6.

43. Wang XL, Pan LL, Long F, Wu WJ, Yan D, Xu P, et al. Endogenous Hydrogen Sulfide Ameliorates NOX4 Induced Oxidative Stress in LPS-Stimulated Macrophages and Mice. Cellular Physiology and Biochemistry. 2018;47(2):458-74.

44. Li L, Salto-Tellez M, Tan C-H, Whiteman M, Moore PK. GYY4137, a novel hydrogen sulfide-releasing molecule, protects against endotoxic shock in the rat. Free Radical Biology and Medicine. 2009;47(1):103-13.

45. Zhuang R, Guo L, Du J, Wang S, Li J, Liu Y. Exogenous hydrogen sulfide inhibits oral mucosal wound-induced macrophage activation via the NF-κB pathway. Oral Diseases. 2018;24(5):793-801.

46. Huang CW, Feng W, Peh MT, Peh K, Dymock BW, Moore PK. A novel slow-releasing hydrogen sulfide donor, FW1256, exerts anti-inflammatory effects in mouse macrophages and in vivo. Pharmacological Research. 2016;113:533-46.

47. Miao L, Shen X, Whiteman M, Xin H, Shen Y, Xin X, et al. Hydrogen Sulfide Mitigates Myocardial Infarction via Promotion of Mitochondrial Biogenesis-Dependent M2 Polarization of Macrophages. Antioxidants & Redox Signaling. 2016;25(5):268-81.

48. Zhang Q, Yuan L, Liu D, Wang J, Wang S, Zhang Q, et al. Hydrogen sulfide attenuates hypoxia-induced neurotoxicity through inhibiting microglial activation. Pharmacological Research. 2014;84:32-44.

49. Velmurugan GV, Huang H, Sun H, Candela J, Jaiswal MK, Beaman KD, et al. Depletion of H2S during obesity enhances store-operated Ca2+ entry in adipose tissue macrophages to increase cytokine production. Science signaling. 2015;8(407):ra128-ra.

50. Du H-P, Li J, You S-J, Wang Y-L, Wang F, Cao Y-J, et al. DNA methylation in cystathionine-γ-lyase (CSE) gene promoter induced by ox-LDL in macrophages and in apoE knockout mice. Biochemical and Biophysical Research Communications. 2016;469(3):776-82.

51. Wang X-H, Wang F, You S-J, Cao Y-J, Cao L-D, Han Q, et al. Dysregulation of cystathionine γ-lyase (CSE)/hydrogen sulfide pathway contributes to ox-LDL-induced inflammation in macrophage. Cellular Signalling. 2013;25(11):2255-62.

52. Li J-J, Li Q, Du H-P, Wang Y-L, You S-J, Wang F, et al. Homocysteine Triggers Inflammatory Responses in Macrophages through Inhibiting CSE-H2S Signaling via DNA Hypermethylation of CSE Promoter. International journal of molecular sciences. 2015;16(6):12560-77.

53. Bronowicka-Adamska P, Jurkowska H, Gawda A, Skalska P, Nazimek K, Marcinkiewicz J, et al. Expression and activity of hydrogen sulfide generating enzymes in murine macrophages stimulated with lipopolysaccharide and interferon-γ. Molecular Biology Reports. 2019.

54. George L, Ramasamy T, Sirajudeen KNS, Manickam V. LPS-induced Apoptosis is Partially Mediated by Hydrogen Sulphide in RAW 264.7 Murine Macrophages. Immunological Investigations. 2019:1-15.

55. Castelblanco M, Lugrin J, Ehirchiou D, Nasi S, Ishii I, So A, et al. Hydrogen sulfide inhibits NLRP3 inflammasome activation and reduces cytokine production both in vitro and in a mouse model of inflammation. The Journal of biological chemistry. 2018;293(7):2546-57.

56. Yue L-M, Gao Y-M, Han B-H. Evaluation on the effect of hydrogen sulfide on the NLRP3 signaling pathway and its involvement in the pathogenesis of atherosclerosis. Journal of Cellular Biochemistry. 2019;120(1):481-92.

57. Tang X-Q, Fan L-L, Li Y-J, Shen X-T, Zhuan Y-Y, He J-Q, et al. Inhibition of Hydrogen Sulfide Generation Contributes to 1-Methy-4-Phenylpyridinium Ion-Induced Neurotoxicity. Neurotoxicity Research. 2011;19(3):403-11.

58. Ghanbari F, Khaksari M, Vaezi G, Hojati V, Shiravi A. Hydrogen Sulfide Protects Hippocampal Neurons Against Methamphetamine Neurotoxicity Via Inhibition of Apoptosis and Neuroinflammation. Journal of Molecular Neuroscience. 2019;67(1):133-41.

59. Ji J, Xiang P, Li T, Lan L, Xu X, Lu G, et al. NOSH-NBP, a Novel Nitric Oxide and Hydrogen Sulfide-Releasing Hybrid, Attenuates Ischemic Stroke-Induced Neuroinflammatory Injury by Modulating Microglia Polarization. Frontiers in cellular neuroscience. 2017;11:154-.

60. Zhao Z-Z, Wang Z, Li G-H, Wang R, Tan J-M, Cao X, et al. Hydrogen sulfide inhibits macrophage-derived foam cell formation. Experimental Biology and Medicine. 2011;236(2):169-76.

61. Qin M, Long F, Wu W, Yang D, Huang M, Xiao C, et al. Hydrogen sulfide protects against DSS-induced colitis by inhibiting NLRP3 inflammasome. Free Radical Biology and Medicine. 2019;137:99-109.

62. Zhang D, Du J, Tang C, Huang Y, Jin H. H2S-Induced Sulfhydration: Biological Function and Detection Methodology. Frontiers in Pharmacology. 2017;8:608.

63. Short JD, Downs K, Tavakoli S, Asmis R. Protein Thiol Redox Signaling in Monocytes and Macrophages. Antioxidants & redox signaling. 2016;25(15):816-35.

64. Barrett WC, DeGnore JP, König S, Fales HM, Keng Y-F, Zhang Z-Y, et al. Regulation of PTP1B via Glutathionylation of the Active Site Cysteine 215. Biochemistry. 1999;38(20):6699-705.

65. Kim HS, Asmis R. Mitogen-activated protein kinase phosphatase 1 (MKP-1) in macrophage biology and cardiovascular disease. A redox-regulated master controller of monocyte function and macrophage phenotype. Free radical biology & medicine. 2017;109:75-83.

66. Kim HS, Ullevig SL, Zamora D, Lee CF, Asmis R. Redox regulation of MAPK phosphatase 1 controls monocyte migration and macrophage recruitment. Proceedings of the National Academy of Sciences of the United States of America. 2012;109(41):E2803-E12.

67. O'Neill Luke AJ. A Metabolic Roadblock in Inflammatory Macrophages. Cell Reports. 2016;17(3):625-6.

68. Rodríguez-Prados J-C, Través PG, Cuenca J, Rico D, Aragonés J, Martín-Sanz P, et al. Substrate Fate in Activated Macrophages: A Comparison between Innate, Classic, and Alternative Activation. The Journal of Immunology. 2010;185(1):605.

69. Vats D, Mukundan L, Odegaard JI, Zhang L, Smith KL, Morel CR, et al. Oxidative metabolism and PGC-1β attenuate macrophage-mediated inflammation. Cell Metabolism. 2006;4(1):13-24.

Figure Legends

Figure 1. The regulatory mechanisms underlying H_2S signaling. The mechanisms underlying H_2S signaling in the regulation of macrophage function involve direct mediation of protein S-sulfhydration, cellular redox homeostasis and epigenetic chromatin remodeling.

Figure 2. H_2S signaling regulates macrophage functionality for the initiation and resolution of an inflammatory response. Upon stimulation (e.g., LPS and oxLDL), H_2S production is shut down at the early stage to facilitate pro-inflammatory cytokine secretion, while at the late stage, the H_2S content becomes increased for induction of those mission-completed macrophages to undergo apoptosis. Alerted H_2S signaling would lead to the development of immune or metabolic disorders.