Biochimica et Biophysica Acta 1820 (2012) 763–769

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Review

S-nitrosylation of surfactant protein D as a modulator of pulmonary inflammation ☆

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ARTICLE INFO

Article history:
Received 20 April 2011
Received in revised form 13 October 2011
Accepted 4 December 2011
Available online 13 December 2011

Keywords:
SP-D, Surfactant protein D
TLR, Toll like receptor
NO, Nitric oxide
SNO, *S*-nitrosothiol
Pulmonary inflammation
Macrophages polarization

ABSTRACT

Background: Surfactant protein D (SP-D) is a member of the family of proteins termed collagen-like lectins or "collectins" that play a role in non-antibody-mediated innate immune responses [1]. The primary function of SP-D is the modulation of host defense and inflammation [2].
Scope of review: This review will discuss recent findings on the physiological importance of SP-D *S*-nitrosylation in biological systems and potential mechanisms that govern SP-D mediated signaling.
Major conclusions: SP-D appears to have both pro- and anti-inflammatory signaling functions.
SP-D multimerization is a critical feature of its function and plays an important role in efficient innate host defense. Under baseline conditions, SP-D forms a multimer in which the N-termini are hidden in the center and the C-termini are on the surface. This multimeric form of SP-D is limited in its ability to activate inflammation. However, NO can modify key cysteine residues in the hydrophobic tail domain of SP-D resulting in a dissociation of SP-D multimers into trimers, exposing the *S*-nitrosylated N-termini. The exposed *S*-nitrosylated tail domain binds to the calreticulin/CD91 receptor complex and initiates a pro-inflammatory response through phosphorylation of p38 and NF-κB activation [3,4]. In addition, the disassembled SP-D loses its ability to block TLR4, which also results in activation of NF-κB.
General significance: Recent studies have highlighted the capability of NO to modify SP-D through *S*-nitrosylation, causing the activation of a pro-inflammatory role for SP-D [3]. This represents a novel mechanism both for the regulation of SP-D function and NO's role in innate immunity, but also demonstrates that the *S*-nitrosylation can control protein function by regulating quaternary structure. This article is part of a Special Issue entitled Regulation of Cellular Processes by *S*-nitrosylation.

1. Introduction

The alveolar-lining interface is continuously exposed to a wide range of inhaled pathogens from the external environment [1]. The pulmonary innate immune system creates a unique balance between a protective response against harmful pathogens and an appropriate inflammatory response partially via generation of reactive chemical species [2,5]. NO plays an important role in the regulation of inflammation within the innate immune system via reaction with a range of biological targets. There is increasing evidence that *S*-nitrosothiols form a principle mechanism of transducing NO bioactivity and regulating protein function [3]. However, the biology of *S*-nitrosothiol signaling within pulmonary inflammation remains largely unexplored. The present review discusses current knowledge on how NO serves to regulate the pulmonary collectin, SP-D, a key controller of the innate immune system. There is a focus on how *S*-nitrosylation of the N-terminal cysteines of SP-D regulates lung inflammation.

2. *S*-nitrosylation as a regulator of lung inflammation through TLR4 signaling pathway

Toll-like receptors (TLRs) are family of pattern recognition receptors that represent the first line of defense against many pathogens. Remarkably, TLRs are capable of discriminating between pathogenic and commensal microorganisms, and inducing appropriate and distinct antimicrobial response [5]. As regulators of the inflammatory response against pathogens, TLRs help to strengthen the processes of innate and adaptive immunity [6].

In mammals, the recognition of lipopolysaccharide (LPS) requires at least three proteins: TLR4, CD14 and myeloid differentiation factor

Abbreviations: TLR, Toll-like receptor; TIR, Toll/IL-1 receptor domain; LPS, lipopolysaccharides; LBP, LPS-binding protein; MD-2, myeloid differentiation factor 2; MyD88, myeloid differentiation protein 88; IRAK, IL-1 receptor associated kinase; TRAF6, TNF receptor associated factor; MAPK, mitogen-activated protein kinase; TAK1, transforming growth factor b-associated kinase 1; TAB1, TAK1-binding protein 1; TAB2, TAK1-binding protein 2; IKK, IκB kinase; IκB, inhibitor of NF-κB; NF-κB, nuclear factor kappa B; SIRP-1α, signal inhibitory regulatory protein-1α; SHP-1, tyrosine-protein phosphatase-1; MBL, mannose-binding lectin; ARDS, acute respiratory distress syndrome; ALI, acute lung injury; COPD, chronic obstructive pulmonary disease

☆ This article is part of a Special Issue entitled Regulation of Cellular Processes by *S*-nitrosylation.

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doi:10.1016/j.bbagen.2011.12.006

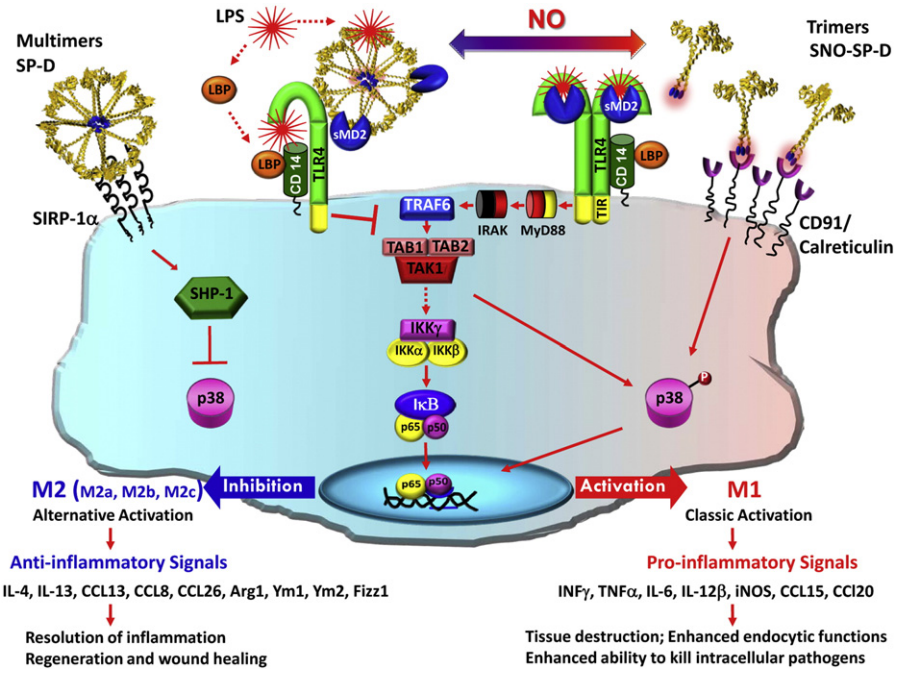

Fig. 1. The "Dandelion Ball Model" of the immunomodulatory functions of SP-D in macrophages. Under baseline conditions the hydrophobic N-terminus of SP-D is hidden in the center of the "dandelion ball" with exposed carboxy terminal domains outside. Numerous studies have shown that CRD-specific interactions of SP-D with immune cells may be mediated through a number of receptors. It has been proposed that binding of SP-D "dandelion ball" via its CRD domains with SIRP-1α induces the activation of tyrosine phosphatase SHP-1, resulting in blockage of the downstream signaling through p38 MAP kinase [51]. The interaction of CRD domain of SP-D "dandelion ball" with sCD14, sMD2 and TLR4 may block the binding of LPS to these receptors leading to inhibition of NF-κB activation [44–46]. Potentially, this may be achieved by SP-D inhibiting TLR4 dimerization in the presence of LPS. Under pro-inflammatory circumstances *S*-nitrosylation of cysteine residues in the hydrophobic tail domain of SP-D results in disruption of multimeric SP-D structure (disassembling of a "dandelion ball") and exposure of *S*-nitrosylated tail domain [3]. The exposed *S*-nitrosylated tail domain is now available to bind with the calreticulin/CD91 receptor complex and initiates a pro-inflammatory response through phosphorylation of p38 and NF-κB activation. [3,4]. In addition, within this model one can see that the disassembled "dandelion ball" of SP-D would have lost its ability to block LPS mediated activation of NF-κB. In the absence of multimeric SP-D, or in its absence, LPS forms a CD14–MD2–TLR4 complex which triggers TLR4 dimerization and recruitment of the adaptor protein, MyD88. Dimerization results in IRAK phosphorylation and oligomerization of TRAF6 assembled TRAF6–TAK1–TAB1–TAB2 complex is translocated to the cytosol where it phosphorylates IκB through activation of IKK and therefore results in translocation of NF-κB to the nucleus with the consequent transcription of immune response genes [8–11,45].

2 (MD-2) [7] (Fig. 1). LPS is opsonized by LPS-binding protein (LBP), and the complex is recognized by the opsonic receptor CD14 on the macrophage surface and forms the ternary complex LPS–LBP–CD14. Transfer of LPS from CD14 to MD-2 is tied to binding to TLR4 [8]. Lipid chains of LPS shift into the hydrophobic pocket of MD-2 to maximize hydrophobic contact. This structural shift allows the phosphate groups of LPS to form ionic interactions with a cluster of positively charged residues in TLR4 and MD-2 and this, in turn, results in dimerization of the two LPS–MD-2–TLR4 complexes arranged symmetrically [9]. TLR dimerization results in the recruitment of the adaptor protein, MyD88. The intracellular of TLR4 (TIR) binds to a homologous domain in MyD88 to constitute the so-called "death domain". This death domain undergoes homophilic interaction with a similar domain of the serine/threonine protein kinase, IRAK, resulting in IRAK phosphorylation. Auto-phosphorylated IRAK forms a complex with TRAF6, which initiates oligomerization of TRAF6 [10]. This results in association with the heterotrimer of TAK1–TAB1–TAB2 to form a multi-component complex. Upon phosphorylation of TAK1 and TAB2, IRAK dissociated from the complex, and the TRAF6–TAK1–TAB1–TAB2 complex is translocated to the cytosol where it activates IKK [10]. IKK phosphorylates IkB, leading to its proteolytic degradation and the translocation of NF-κB to the nucleus with the consequent transcription of immune response genes [11].

There is a considerable experimental evidence indicating that TLR signaling cascade contains a number of proteins, including MyD88, IKKβ and NF-κB, whose activity is regulated via *S*-nitrosylation [12]. It has been shown that *S*-nitrosylation of the p50–p65 heterodimer inhibits its binding to iNOS promoter DNA at the NF-κB–DNA interface [13,14]. *S*-nitrosylation of the IkB kinase β, a major regulator of NF-κB, results in reduction of its kinase function, which in turn leads to a reduction in IkB ubiquitinylation and subsequent degradation [15]. *S*-nitrosylation of the adaptor protein MyD88, located upstream from the IKK complex, results in inhibition of its binding to the sorting adaptor TIRAP, and therefore decreases LPS-induced NF-κB activation [16]. All these studies demonstrate that *S*-nitrosylation inhibits TLR signaling through modification of components of the intracellular signaling cascade. Therefore, one can see that the generation of intracellular SNO is an important mechanism in the control of acute-phase inflammatory responses.

3. SP-D as a target for *S*-nitrosylation

SP-D is a Ca^{2+}-binding lectin that is produced primarily as a multimer by alveolar type II cells and nonciliated bronchiolar cells in the lung [1,17]. SP-D shares considerable structural homology with other proteins of this type, including surfactant protein A (SP-A), conglutinin, bovine collectin-43, and mannose binding protein [18,19]. SP-D through its C-type carbohydrate-recognition domain (CRD) binds to carbohydrate structures present on a range of viruses, bacteria, yeasts and fungi [20,21]. Upon recognition of the infectious agents, SP-D initiates direct opsonization, neutralization, agglutination and phagocytosis [2,22]. In addition, it can interact with receptor molecules present on immune cells leading to enhanced microbial clearance and modulation of inflammation [23,24]. Targeted ablation of the SP-D gene results in chronic inflammation and an emphysema-like phenotype in the absence of infection [25,26]. The lungs of SP-D (−/−) mice are characterized by an inappropriate activation of alveolar macrophages with significant alterations in nitric oxide (NO) metabolism [27–30], indicating an important role for SP-D in regulating immune homeostasis and