Soybean lectin-induced autophagy follows P2RX7 activated NF-κB-ROS pathway to kill intracellular mycobacteria

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ABSTRACT

Host-directed therapy has now been accepted as a novel anti-TB strategy to ameliorate the ever threatening global tuberculosis (TB) burden. In past years, autophagy is one of such strategies that has been manipulated through various inducers to curtail the intracellular Mycobacterium tuberculosis (M. tb) survival. Amongst various inducers, triggering autophagy through natural compounds is a treasure resource whose potential can be harnessed for better therapeutics against TB. In this study, we investigated the antimycobacterial role of soybean lectin (SBL), a legume lectin and mechanistic interplay of autophagy is this response. We first performed time kinetic experiment with the non-cytotoxic dose of SBL (20 μg/ml) in PMA differentiated THP-1 (dTHP-1) cells and observed optimum expression of major autophagic markers like autophagy related genes (Atg) 7, Atg3 and elevated LC3 puncta formation after 24 h of treatment. We further validated this autophagy induction through MDC staining where SBL-treated cells showed more accumulation of autophagosomes in comparison to the control cells. Abrogation of autophagy in the presence of 3-MA and increase in LC3 puncta formation upon Baf-A1 addition clearly elucidated the specific effect on autophagy and autophagic flux in SBL treated dTHP-1 cells. SBL treatment also led to autophagy induction in mycobacteria (M. smegmatis and M. bovis BCG) infected macrophages that restrained the intracellular mycobacterial growth thus emphasizing the host defensive role of SBL induced autophagy. To understand the mechanism of antimycobacterial effect through autophagy, we found increased P2RX7 expression, NF-κB activation and reactive oxygen species (ROS) generation in dTHP-1 cells upon SBL treatment. Inhibition of P2RX7 expression either by KN-62 (P2RX7 antagonist) or P2RX7 siRNA blocked p65 nuclear translocation thereby suppressing NF-κB dependent ROS level in SBL treated dTHP-1 cells. Moreover, SBL-induced autophagy was abrogated in the presence of different inhibitors like KN-62(NF-κB inhibitor/N-acetyl cysteine (NAC)) and P2RX7 siRNA leading to more survival of intracellular mycobacteria. Taken together, these results conclude that SBL induced autophagy exerts antimycobacterial effect in P2RX7-NF-κB dependent manner through ROS generation.

INTRODUCTION

M. tb is a successful human pathogen that primarily infects lungs and resides in alveolar macrophages. Macrophages act as principal reservoirs of M. tb and as part of innate immune defence, they try to kill the bacteria thereby clearing the infection at primary stage. However, M. tb employs different strategies to avoid host defence mechanisms like inhibiting antigen presentation, apoptosis, generation of reactive oxygen species and nitrogen intermediates and autophagy. From previous study, it has been reported that induction of autophagy inside macrophages compels M. tb loaded phagosome to fuse with the lysosome resulting in killing of bacteria. Published reports have also shown that various autophagy inducing molecules like gefitinib, vitamin D, mTOR inhibitors, metformin, statins, verapamil, fluoxetine, nitroimidazole, carbamazepine, calcinein, 5-Nitro-1,10-phenanthroline and valproic acid imposed detrimental effect on the intracellular survival of mycobacteria. Further, antimycobacterial action of IP-10 is reported to be associated with induction of autophagy in infected host cell. So, in this line, natural substances such as plant derived products or pharmacological agents that can stimulate autophagy in infected cells can be of great importance in treating tuberculosis. One of such class of plant protein is Soybean lectin (SBL) which have been shown to possess anticancer activity mediated through induction of autophagy. Therefore, the present study was undertaken to study the antimycobacterial effect of SBL through autophagy.

METHODS

PMA differentiated THP-1 cells (dTHP-1 cells)
Infected with either M. smegmatis or M. bovis BCG
Expression of autophagic markers, LC3 puncta and autophagic vacuole accumulation
Expression of P2RX7 and its binding to SBL
Activation of NF-κB upon SBL binding to P2RX7
ROS generation following NF-κB activation
Effect of different inhibitors like KN-62, NF-κB inhibitor IV or NAC on autophagy induction and intracellular mycobacteria survival

RESULTS

Fig. 1. Effect of varying concentrations of SBL on cell viability of THP-1 cells after 72h of treatment

Fig. 2. Effect of non-cytotoxic concentration of SBL (20 μg/ml) on autophagic markers expression in dTHP-1 cells at different time points and autophagic vacuole accumulation after 24h of treatment

Fig. 3. Effect of SBL on autophagic flux in SBL treated dTHP-1 cells

Fig. 4. Effect of SBL on autophagy in M. smegmatis infected dTHP-1 cells and its repression on intracellular M. smegmatis (C) and M. dourae BCG (D) viability

Fig. 5. P2RX7 expression at mRNA and cell surface levels upon SBL treatment, its binding with SBL and effect of SBL on P2RX7 dependent NF-κB activation in dTHP-1 cells

Fig. 6. Effect of either KN-62 or NF-κB inhibitor on autophagic response in SBL treated dTHP-1 cells

Fig. 7. Effect of SBL treatment on ROS generation, effect of either KN-62 or NF-κB inhibitor on SBL induced ROS level, effect of ROS scavenger on autophagic response in SBL treated dTHP-1 cells and inhibition of P2RX7/NF-κB-ROS pathway on intracellular mycobacterial survival

CONCLUSIONS

1. Non-toxic concentration of SBL induced autophagy in both uninfected and mycobacteria infected dTHP-1 cells
2. 3-MA and Baf-A1 abrogated the autophagy induction potential of SBL
3. SBL induced autophagy decreased the intracellular viability of mycobacteria.
4. SBL treatment led to elevated expression of P2RX7, NF-κB activation and increased ROS generation.
5. Inhibition of P2RX7 resulted in inhibition of NF-κB activation leading to reduction in ROS generation.