Bacillus parabiotic modulates TLR3 and ACE2 gene expressions in RAW264.4 cells induced by viral mimetic Poly I:C

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Abstract: *Background*: Toll-like receptor-3 (TLR3) induces a double-stranded RNA (dsRNA)-mediated inflammatory signal in the cells of the innate immune system. Angiotensin-converting enzyme 2 (ACE2) has been established as the functional host receptor for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and induced through TLR3 signals .Simultaneously, the interaction between TLR3, ACE2 and SARS-CoV-2 increases the secretion of soluble ACE2 in the blood and leading to the release of a massive quantity of cytokines (cytokine storm). Here we investigated the possible application of Bacillus parabiotics modulates TLR3 and ACE2 gene expressions in RAW264.4 cells induced by viral mimetic Poly I:C. *Method*: The murine macrophage cell line RAW264.7 maintained in Dulbecco's modified Eagle medium containing 10% (v/v) FCS. RAW264.7 cells (1 × 105 cells /well) were plated in fresh medium in 6 well plates overnight. Inactivated Bacillus probiotic (parabiotic) was obtained by autoclaving bacterial cell pellets (121 ºC, 15min). RAW264.7 cells were exposed to spore parabiotic Bacillus sp. 1630F (1 × 106 CFU/mL) for 3 hrs. The poly I:C (10 µg/mL) were added to wells and kept for further for 3 hrs. Cell lines only treated by poly I:C were used as inflammatory control. Total RNA from flash-frozen Cells (with liquid nitrogen) was isolated using the Yekta kit (Tehran, Iran) in accordance with the manufacturer's instructions. DNase treated RNA was transcribed into complementary DNA (cDNA) using cDNA synthesis Kit (Invitrogen, Carlsbad, USA) following the manufacturer's instructions. qRT-PCR was performed against some putative TLR3 and ACE2 genes (Applied Biosystems Co, California, USA). The Act1 (beta-actin) gene was used as the internal control, and gene expressions were calculated using the formula 2−ΔΔCT while all primer pairs displayed similar amplification efficiency. *Result*: We assessed the immunomodulating capacity of heat killed b.1630F on RAW264.7 cell line by examining its effect on expression of ACE2 and TLR3.The result shown that treatment with Poly:IC induced the expression of TLR3 and ACE2. However the expression of TLR3 and ACE2 gene was decreased 126- and 8.5 folds in the presence of parabiotic strain. *Discussion*: Our bacillus parabiotic cells showed anti-inflammatory effects on RAW264.4 cells by modulating TLR3 signaling pathway and ACE2

receptor following the inflammation induction by PolyI:C. Therefore Bacillus parabiotic as a through intra-nasal administration can both prevent and reduce inflammation-related diseases such as SARS-CoV-2. ***Keywords***: Parabiotic, Bacillus, Inflammation, TLR3, ACE2