

Expression of TLR4 mRNA in lungs of mice after single and multiple exposures to poultry barn air

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ABSTRACT

The poultry sector in India being a commercial activity requires workers to spend more number of hours inside the barns. The exposure of workers to barn air may also increase susceptibility to secondary microbial challenges. However, there are no data on the health effects of exposure to the barn from tropical climates. Because of the central role of the toll like receptor 4 (TLR-4) in endotoxin-induced lung inflammation, we evaluated expression of TLR-4 following single and multiple exposures to poultry barn air followed by lipopolysaccharides (LPS) challenge. Male mice (80) aging 7-8 weeks were divided into 4 groups, viz. 3 treatments and 1 control (20; each). Among the treatment groups, Group 1 was exposed to poultry barn air for single day, group 2 for 6 days (Mon to Sat) and group 3 for 24 days (4 weeks, Mon to Sat) for 8 hrs. At the end of exposure period, half of the animals (10) from each group were challenged with LPS @ 100µg/ animal. Immediately following sacrifice, the lung tissues were collected to study the expression profile of TLR-4 mRNA by quantitative RT-PCR. Single exposure did not show any significant change in the expression of TLR-4 mRNA, while 6 days multiple exposures resulted in almost 6 fold increase in the expression of TLR-4 mRNA. There was no change in the expression of TLR-4 mRNA after 24 days multiple exposures compared to control suggesting that prolong multiple exposures may induce adaptation. However, single and 6 and 24 days multiple exposures followed by LPS challenge showed 6, 47 and 17 fold increase in the expression of TLR-4 mRNA, respectively. The data showed exposure to poultry barn air alters TLR-4 expression which may underlie altered responsiveness to LPS challenge.

Key words: Lungs, Multiple exposures, Poultry barn air, Single exposure, TLR4

The increase in poultry production has created employment for farm workers, however, this employment came along with certain health problems particularly respiratory disorders (Simpson *et al.* 1998) and chicken barn workers appear to be the most susceptible to various respiratory problems (Just *et al.* 2009). Lung function in general are lower in poultry barn workers and a number of these changes are associated with dust and/or endotoxins levels in these facilities (Kirychuk *et al.* 2006). Many inflammatory molecules and endotoxin bind to toll like receptors (TLRs) and had been shown to contribute to lung inflammation in the workers. A variety of cells including macrophages and neutrophils express TLR4 for recognition and phagocytosis of bacteria (Latz *et al.* 2004). The ligation of TLR4 leads to the activation of macrophages, production of cytokines and chemokines and subsequent recruitment of inflammatory cells including pro-inflammatory mediators (Andonegui *et al.* 2003). The interaction between TLR4 and lipopolysaccharides (LPS) results in an increased Th₁ immune response. However, the role of TLR4 and

bacterial endotoxins in respiratory dysfunctions has not been tested in an experimental model. The present study was carried out to detect expression profile of TLR4 in lungs of mice after single and multiple exposures to poultry barn air.

MATERIALS AND METHODS

The experiment protocols were approved by Institutional Animal Ethics Committee, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. The animals were purchased from Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar and kept at small animal house for 1 week for acclimatization. Healthy Swiss albino male (80) mice aging 7-8 weeks were randomly divided into 4 groups, viz. 3 treatments and 1 control group (20; each). The animals from all the groups were transported to poultry farm in cages and were exposed to poultry barn air for 8 h daily (9 AM to 5 PM) and were brought back to small animal house after exposure. Group 1 was exposed for a single day, group 2 for 6 days (Mon to Sat) and group 3 for 24 days (4 weeks from Mon to Sat except Sun). There was cage-housing system in poultry farm and animals were kept in cages at height of 3 feet inside the poultry barn. At the end of exposure, 10 mice from each group were

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administered LPS @ 100µg/ animal and remaining with normal saline solution (NSS) via intraperitoneal route (Barsness *et al.* 2004). The animals with NSS served as control to the respective group. After 9 h of LPS/NSS challenge mice were humanly sacrificed with xylazine ketamine anesthesia. The thoracic cavity of mice was opened and lungs were collected and stored in RNA Later solution. The lung samples in RNA Later solution were subjected to Quantitative RT-PCR (qPCR) analysis.

RNA isolation and cDNA synthesis: Total RNA from lung stored in RNA later solution was isolated using TRIZOL method and optical density of nucleic acid was measured in an ultra violet light Nanodrop spectrophotometer. The RNA was reverse transcribed using first-strand cDNA synthesis kit as per manufacturer's instructions.

Quantitative RT-PCR (qPCR): The cDNA from lung tissue was used for quantitative RT-PCR analysis for expression of TLR4 gene using SYBR Green PCR kit as per manufacturer's instructions. The β -Actin was used as reference housekeeping gene. The reaction was performed using primer pair: 5'-TGCTGAGTTTCTGATCCATGC-3' and 3' TGGCTAGGACTCTGATCATGG- 5' for TLR4 (Zuo *et al.* 2012) and 5'- GCACCACACCTTCTACAATG-3' and 3'-TGCTTGCTGATCCACATCTG-5' for β -Actin (Guo *et al.* 2013). The qPCR was performed using kit. Results were standardized to control-exposed animals and given as relative fluorescence over control mRNA level (fold difference) after correction for expression of β -Actin. The reaction was run in duplicates.

RESULTS AND DISCUSSION

There was no increase in the expression of TLR4 mRNA following single exposure to poultry barn air but single exposure followed by LPS challenge resulted 6 folds increase in expression of mRNA. (Figs 1-2). LPS is major cell wall components of the outer membrane of Gram-negative bacteria, which induces systemic inflammation and is a major pathogenic element (Sugiyama *et al.* 2008). TLR4 is a critical LPS sensor to induce cell signalling and production of inflammatory mediators (Bowie *et al.* 2000; Flo *et al.* 2001).

Multiple exposures of 6 days caused nearly 6 folds increase in the expression of TLR4 mRNA and when

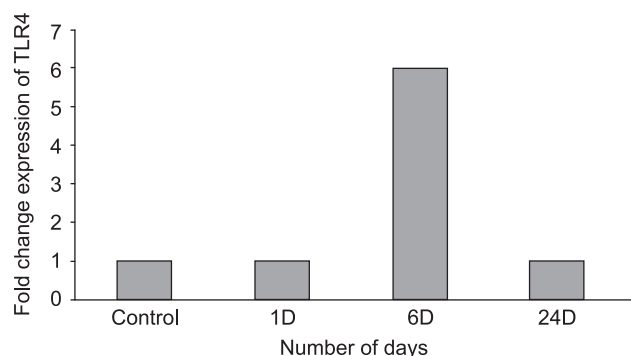


Fig. 1. Expression of TLR4 mRNA single and multiple exposure to poultry barn air.

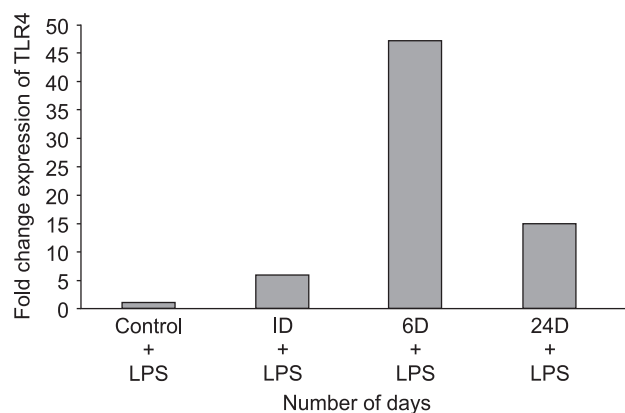


Fig. 2. Expression of TLR4 mRNA single and multiple exposure to poultry barn air and followed by LPS challenge.

exposure was combined with LPS there was 47 fold increase in mRNA expression (Graph. 1-2). In addition to LPS, TLR4 can bind other ligands, such as various small molecules as well as endogenous or exogenous proteins (Bryant 2010). LPS triggers the physical interaction between CD14 and TLR4 that mediates LPS signalling pathway and regulates alternative splicing of nuclear factor-kB (NF-kB) in the lungs after injury (Ling *et al.* 2013). Particles of respirable size remain airborne longer and penetrate deeper within the respiratory system (Just *et al.* 2009) suggesting that the higher concentrations of smaller dust particles in cage-housed facilities may be responsible for the more damage to lungs in terms of expression of TLR 4 in the present study. Furthermore, multiple exposures of 24 days resulted in down-regulation of TLR 4 mRNA which suggests adaptation responses.

We came up with conclusion that single exposure to poultry barn air followed by LPS challenge resulted increased expression of TLR4 mRNA. Multiple exposures of 6 days led to almost 6 fold increase in expression of TLR4 mRNA and 6 days exposure followed by LPS challenge resulted 47 fold increase in expression of TLR4 mRNA. Further, multiple exposures of 24 days resulted down-regulation of TLR 4 mRNA compared to 6 days multiple exposures suggesting that prolonged multiple exposures may induce adaptation in the exposed mice. These changes suggested that exposures to poultry barn air alter TLR4 expression and exposure followed by LPS challenge may modulate the pulmonary responsiveness to such exposures.

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