Identification of Nuclear Factor-κB Pathway Genes in Chicken Erythrocytes and their Expression Level in Erythrocytes after Infection with Mycoplasma synoviae

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ABSTRACT

Mycoplasma synoviae is one of the most important pathogens in the poultry industry and often causes diseases of a chronic and persistent nature. However, there is limited data available on Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathway genes expression in chicken erythrocytes infected with Mycoplasma synoviae (M. synoviae). Therefore, the aim of the current in-vitro study was to determine the interaction between chicken erythrocytes and M. synoviae using Transmission electron microscope (TEM) and further to investigate the mRNA gene expression of MyD88 (Myeloid differentiation primary response 88), CCL5 (C-C Motif Chemokine Ligand 5), MDA5 (melanoma differentiation-associated protein 5), IKBKE (inhibitor of nuclear factor kappa-B kinase subunit epsilon), NFKBIA (NF-kappa-B inhibitor alpha), NFKBIE (NF-kappa-B inhibitor epsilon), Interferon Alpha (IFN-α), cMGF (chicken myelomonocytic growth factor), and TRAF6 (Tumor necrosis factor receptor-associated factor 6) in chicken erythrocytes infected with M. synoviae using quantitative real-time PCR (qRT-PCR) at different time intervals such as 0 h, 2 h and 6 h while later on the expression level significantly increased. These results will lead to increased insights on NF-κB signaling pathway and other immune system genes in the control of the host immune response.

INTRODUCTION

Erythrocytes in circulation are the most abundant cells which plays critical role in the exchange and transportation of gases. In the blood, erythrocytes are the key bactericidal cells, which perform pathogens clearance in bloodstream. They attract, engulf, kill and then push killed microorganisms back to blood plasma (Minasyan, 2014). Furthermore, many studies stated that in immunology, an important role is played by chicken erythrocytes (Paolucci et al., 2013). Indeed, it was shown that candida albicans stimulated erythrocytes released mediators which in turn enhanced phagocytic capabilities after macrophage activation (Passantino et al., 2007). Additionally, in erythrocytes several TLRs transcripts were constitutively expressed (Paolucci et al., 2013).

Mycoplasma synoviae is a significant pathogen of domestic poultry, leads to huge economic losses in poultry industry (Kleven, 2008; Umar et al., 2017). Infection mainly occurs as a subclinical upper respiratory tract infection, but when virulent M. synoviae strains are...
involved along with other respiratory pathogens, may
lead to respiratory disease with air saculitis (Lockaby et al., 1999). Whereas, acute to chronic infectious synovitis can occur as a result of systemic infection in poultry birds (Kleven., 2008). Most importantly, the birds remain carriers for life after being persistently infected with M. synoviae (Raviv et al., 2007; Umar et al., 2017). It has been reported that several immune genes are modulated in response to M. synoviae infection in chicken macrophages (Lavrič et al., 2007; 2008).

Innate immunity is the first line of defense against invading pathogens. After TLRs detect the microbial components, they recruit adaptor proteins to active the NF-kB pathway (Kawai and Akira, 2007). Nuclear factor-kB (NF-kB) represents a family of inducible transcription factors, which regulates a large array of genes involved in different processes of the immune and inflammatory responses (Oeckinghaus et al., 2009) NF-kB induces the expression of various pro-inflammatory genes, including those encoding cytokines and chemokines, and also participates in inflammasome regulation. In addition, NF-kB plays a critical role in regulating the survival, activation and differentiation of innate immune cells and inflammatory T cells. Consequently, deregulated NF-kB activation contributes to the pathogenic processes of various inflammatory diseases (Liu et al., 2017).

The activation of NF-kB involves two major signaling pathways, the canonical and non-canonical (or alternative) pathways, both being important for regulating immune and inflammatory responses despite their differences in signaling mechanism (Sun, 2017; Vallabhapurapu and Karin, 2009). The canonical NF-kB pathway responds to stimuli from diverse immune receptors and leads to rapid but transient NF-kB activation (Hayden and Ghosh, 2008; Vallabhapurapu and Karin, 2009; Hu and Sun, 2016). The non-canonical NF-kB pathway regulates important aspects of immune functions, including lymphoid organ development, the cross-priming function of dendritic cells, B cell survival and germinal center reactions, generation and maintenance of effector and memory T cells, and antiviral innate immunity (Sun, 2017). A well-recognized function of NF-kB is regulation of inflammatory responses. In addition to mediating induction of various pro-inflammatory genes in innate immune cells, NF-kB regulates the activation, differentiation and effector function of inflammatory T cells (Lawrence, 2009; Tak and Firestein, 2001). Recent evidence suggests that NF-kB also has a role in regulating the activation of inflammasomes (Sutterwala et al., 2014).

Our earlier studies have reported that in immunity against thiram induced TD chickens, the main role had been played by chicken erythrocytes, which may also have a role in apoptosis (Jahejo et al., 2020b; Tian et al., 2013; Wang et al., 2018). In addition, chicken erythrocytes express TLRs 2, 3, 4, 5, 7 and possess immune related functions (Paolucci et al., 2013). However, till date there was no evidence, whether NF-kB signaling pathway and other immune system genes expressed in M. synoviae infected chicken’s erythrocytes or not and how M. synoviae affects their genes expressions. Therefore, in current study we planned to determine the interaction between M. synoviae and erythrocytes and further to determine the effect of M. synoviae strain on the mRNA expression of NF-kB signaling pathway and other immune system genes in chicken erythrocytes.

MATERIALS AND METHODS

Erythrocyte collection

Blood was obtained from Specific pathogen free (SPF) chickens purchased from Longkol Company (Taigu, Shanxi). In the same volume of Alsever’s solution (Solarbio, Beijing, China), about 4 mL of fresh venous blood from pterygoid vein of adult SPF chicken was drawn and mixed. To the 4 mL Histopaque-1119 solution (Sigma–Aldrich, Oakville, ON), the diluted blood was carefully added following centrifugation at 2000 r/min for 20 min; consequently, the leukocytes and platelets were removed from the supernatant. Later procedures were done as previously described (Kabanova et al., 2009). Moreover, via Wright Giemsa staining 99.9% purity of isolated erythrocytes were determined.

Treatment of Mycoplasma

About 8 mL of FM-4 mycoplasma culture medium was taken during log phase (the medium has just turned yellow). The concentration of mycoplasma was about 1×10^8-1×10^9 mL, followed by centrifugation at 12000 r/min 15min, later supernatant was discarded. After washing twice with PBS again centrifuge at 12000 r/min for 10 min. Lastly cells were than cultured in 98% Dulbecco’s Modified Eagle Medium (DMEM) (Solarbio, Beijing, China), added with 2% fetal bovine serum (FBS) and 2% chicken serum (Longkol, Shanxi, China).

Experimental infection of chicken erythrocytes (CER)

Total 50 µl of erythrocytes were obtained from specific pathogen free (SPF) chickens purchased from Longkol Company (Taigu, Shanxi) in a sixteen 2 mL centrifuge tube containing a certain amount of cell maintenance solution, and distributed into four groups i.e., 0, 2, 6 and 10 h. The experiment was performed in an Animal Biosafety Level 2 Laboratory. To an experimental group, 100 ul of M. synoviae was added into all experimental four groups with the addition of 900 µl of DMEM to make the final volume 1050 µl. Whereas to the another four group M. synoviae was not added and designated as control group.
The cells were then cultured at 37 °C in 5% CO₂ incubator, and each of the respective group was taken out at 0 h, 2 h, 6 h, and 10 h, respectively after the centrifugation for 10 min at 2000 r/min. To the Eppendorf tubes supernatants was drawn and stored. Furthermore, 3 times washing of erythrocytes were done with PBS and stored for upcoming experiments.

**Identification of interaction between M. synoviae and erythrocytes using Transmission Electron Microscope (TEM)**

TEM was performed as described previously (Strunov et al., 2016). Briefly, erythrocytes were isolated at 2000 r/min for 10 min and washed 3 times for transmission electron microscopy. The samples were then fixed and sent to Shanxi Medical University for testing where, the ultrastructure was observed in JEM-1400 (JEOL Ltd., Tokyo, Japan) TEM.

**Total RNA extraction from chicken erythrocytes, and synthesis of cDNA**

From blood samples taken at 0 h, 2 h, 6 h and 10 h from both an experimental and control groups the total RNAs was extracted from erythrocytes that were isolated by RNAiso Plus (Takara Bio Inc., Dalian, China) by following the instructions of the manufacturer, and then dissolved in 20 μL RNase-free water. The extracted RNAs purity and quantity were initially examined through a NanoDrop Bioanalyzer ND1000 (Labtech, Uckfield, UK) and via 1.5% agarose gel electrophoresis. Furthermore, from 500 ng total RNA, the PrimeScript RT reagent Kit with gDNA Eraser (Takara Bio Inc., Dalian, China) was used to reverse transcribe the total RNAs to cDNA according to the procedures recommended by the manufacturer. The cDNA samples were then stored at − 20 °C after diluted at 1:10 in RNase-free water.

**Analysis of mRNA expression of NF-κB signaling pathway and other immune system genes by quantitative real-time PCR (qRT-PCR)**

The qRT-PCR was used for the NF-κB signaling pathway and other immune system genes expression analysis, using real-time RT-PCR kit (TaKaRa SYBR Premix Ex Taq™II (Takara Bio Inc.). Primer Express 3.0 (Applied Biosystems, Foster City, CA, USA) was used to designed primers of TLR’s and other immunity-related genes according to the respective chicken genes coding sequences in NCBI and manufactured by Shanghai Generay Biotech Co., Ltd. (Shanghai, China). Thorough info of the NF-κB signaling pathway and other immune system genes primers and annealing temperature fixed in this experiment along with GenBank accession numbers of the reference sequences are presented in Table I. For qRT-PCR, the thermal cycling parameters used were described previously (Sheng et al., 2018). The expression level of NF-κB

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**Table I. Primer sequences and accession numbers used in quantitative RT-PCR.**

<table>
<thead>
<tr>
<th>Gene targeted</th>
<th>Primers sequences (5′→3′)</th>
<th>Sizes (bp)</th>
<th>Annealing temp.</th>
<th>Accession number</th>
</tr>
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<tbody>
<tr>
<td>NFKBIA</td>
<td>F: CACCAACTACACAGGCGCATA</td>
<td>100</td>
<td>55 °C</td>
<td>NM_001001472.2</td>
</tr>
<tr>
<td></td>
<td>R: TGAAGGCTCCACGGGCAAGT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IKBKE</td>
<td>F: GCAGGCAGGATGAGGAAGTCT</td>
<td>100</td>
<td>55 °C</td>
<td>XM_015299066.2</td>
</tr>
<tr>
<td></td>
<td>R: CGTACGATACCGACTTCATG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NFKBIE</td>
<td>F: CCGGGACACGTTGGTCTCA</td>
<td>100</td>
<td>55 °C</td>
<td>XM_419490.6</td>
</tr>
<tr>
<td></td>
<td>R: CTGGGCTGCTCCAGATAGA</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>IFN α</td>
<td>F: CCACACCTTCTCAAGAACA</td>
<td>100</td>
<td>55 °C</td>
<td>EU367971</td>
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<tr>
<td></td>
<td>R: GCTCTGAGGTGGTAATGT</td>
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<td>MYD88</td>
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<td>55 °C</td>
<td>NM_001030962</td>
</tr>
<tr>
<td></td>
<td>R: AATGACGACACCCATTCTCC</td>
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<tr>
<td>MDA5</td>
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<td>100</td>
<td>55 °C</td>
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<td></td>
<td>R: TCAAGTGTTCTGCACAGACA</td>
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<tr>
<td>CCL5</td>
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<td>55 °C</td>
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<td></td>
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<tr>
<td>TRAF 6</td>
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<td>55 °C</td>
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<td></td>
<td>R: ACACCACCAGAAGGTTAT</td>
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<tr>
<td>cMGF</td>
<td>F: CAATCACGACGCTTTGATT</td>
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<td>55 °C</td>
<td>M85034</td>
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<td></td>
<td>R: GGTGTTGGGAGAGGT</td>
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<td>18SrDNA</td>
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signaling pathway and other immune system genes relative to the 18S rRNA housekeeping gene was calculated by the QuantStudio™ 6 Flex Real-Time PCR System Software (Applied Biosystems, USA).

Statistical analysis

The real time PCR data was calculated using 2−ΔΔCt method. The data obtained between control and experiment groups for each time point post-infection was employed to Two-way ANOVA to perform statistical analysis. All graphs were accomplished using GraphPad Prism 5. Significant differences were measured once the p-value was *$P < 0.05$, **$P < 0.01$ or $P^{***} \leq 0.001$

RESULTS

Interaction between M. synoviae and erythrocytes

For TEM analysis, we have chosen non-infected chicken erythrocytes and infected chicken erythrocytes with M. synoviae. Control erythrocyte was distributed uniform cytoplasm as shown in control section of Figure 1A. But erythrocyte infected with M. synoviae contain endosome as shown in Figure 1B. it was determined that M. synoviae interacted with erythrocyte.

Expression of NF-κB signaling pathway and other immune system genes

The relative mRNA expression levels of NF-κB signaling pathway genes and other immune system genes in chicken erythrocytes following infection with M. synoviae were analyzed and presented in Figure 1 to investigate the degree of effect on gene expression in chicken erythrocytes after interaction with M. synoviae. We found that the expression levels of NF-κB signaling pathway genes and other immune functioning genes were significantly varied at different time intervals such as 0 h, 2 h, 6 h, 10-h post-infection (Hpi). The qRT-PCR results indicated that relative mRNA expression of NFκBIA, IKBKE and TRAF6 were significantly upregulated, whereas IFN-α and cMGF gene expression was significantly downregulated at 0-h -post-inoculation (hpi) ($P < 0.05$), compared to the control group erythrocytes. Moreover, at 2 hpi, the expression of NFκBIA, IKBKE, NFκBIE, MyD88 and MDA 5 were significantly upregulated ($P < 0.05$) while cMGF was significantly downregulated ($P < 0.05$). Relative mRNA expression of NFκBIA, CCL5, MDA5 and cMGF were significantly up-regulated ($P < 0.05$) compared to a control group at 6hpi. In contrast we observed significant downregulation ($P < 0.05$) of IκBKE and IFN-α at 6 hpi. Furthermore, the expression of NFκBIE, CCL5, TRAF6, IFN-α and MDA5 in infected erythrocytes at 10 hpi were also significantly upregulated ($P < 0.05$) while NFκBIA expression was significantly down-regulated ($P < 0.05$) at 10 hpi compared with the control group.

![Fig. 1. Transmission electron Microscopy (TEM) of chicken erythrocytes showing interaction with M. synoviae. Magnification 20000 × or 10000 ×.](image)

DISCUSSION

M. synoviae infection emerges to cause considerable financial loses to worldwide poultry producers. M. synoviae infection controls numerous immune genes in chicken macrophages (Lavrič et al., 2007, 2008) and apoptotic genes in chicken chondrocytes (Dušanić et al., 2012). Recent studies reports stated that chicken erythrocytes actively participated in important aspects of host immunity (Jahejo et al., 2020; Paolucci et al., 2013). Optimal NF-κB activation is initiated by bacterial or viral infections through Toll-like receptors, phorbol 12-myristate 13-acetate, or interferon. This process involves the signal-induced phosphorylation and subsequent ubiquitin-mediated degradation of IκB by the classical IκB kinase (IKK)-dependent pathway (Perkins, 2007; Hayden and Ghosh, 2008;
NF-κB Genes Expression in Infected Chicken Erythrocytes

Fig. 2. The expression pattern of NF-κB signaling pathway and other immune system genes in chicken erythrocytes on 0 h, 2 h, 6 h, 10 h post-infection (Hpi). This experimental study group was as follows: The control group (Con, erythrocytes treated with PBS, n = 3) and the Experiment group (Exp, erythrocytes infected with M. synoviae, n = 3). Expression levels of NF-κB signaling pathway and other immune system genes were relatively calculated to that of 18S rRNA the housekeeping genes via quantitative real-time PCR. SEM (standard error of mean) represented by error bars. Bars with asterisks indicate a significant (*P < 0.05, **P < 0.01, ***P ≤ 0.001) Up or Downregulated relative mRNA expression when compared to uninfected control group.

Karin and Ben-Neriah, 2000). In the current study, for first time it was investigated that chicken erythrocytes constitutively expressed several different NF-κB pathway and other immune system genes (NFKBIA, NFKBIE, IKBKE, CCL5, MD5, MyD88, TRAF6, IFN-α and cMGF) at the transcript level and responded to M. synoviae by up-regulating different genes expression at certain time interval. Therefore, this result offers a novel perspective for poultry health because, targeting such TLRs for therapeutic purposes can be one of the ways to defend chickens from infection of mycoplasmas, particularly, M. synoviae.

There are few reports regarding gene expression of NFKBIA, NFKBIE and IKBKE during bacterial infection. However, genetic variations within NFKBIA, NFKBIE and IKBKE have been shown to influence susceptibility to invasive bacterial infection (Zimmerman et al., 2010). The expression of NFKBIA was significantly upregulated during early h of infection (0 h, 2 h and 6 h) while there was significant downregulated expression at 10 h post infection on M. synoviae infection in chicken erythrocytes. Exposure of cells to various stimulants resulting in release of NF-κB from inhibitor IKB that controls NF-κB activity. Signals activate NF-κB by targeting IKB for proteolysis (Asakrah et al., 2013). An NF-κB dependent host response was shown by the significant differential expression of NFKBIA. Phosphorylation and the subsequent ubiquitination of IKB, the gene product of the NFKBIA gene, are known as key processes required for regulating the innate immune system (Bhoj and Chen, 2009).

Furthermore, our results are in agreement with (Tripathi et al., 2009) who reported that erythrocytes infected with plasmodium Falciparum upregulates the expression of NF-κB pathway genes including NFKBIA, NFKBIE in humans. The kinase was first identified as an IKK kinase family member of the NF-κB activation by lipopolysaccharides and phorbolesters (Shimada et al., 1999; Peters et al., 2000). IKBKE (IKKE, IKKi) is a non-canonical I-kappa-B kinase which can be activated by numerous stimuli (Boehm et al., 2007). IKBKE mainly mediates NF-κB activation induced by the T cell receptor, phorbol 12-myristate 13-acetate, or interferon. In current study we observed dramatic expression pattern differences of the NF-κB related gene such as IKBKE whose expression was significantly upregulated at 0h and 2h while at 10 h post-
infection the expression was significantly downregulated in infected erythrocytes. Previously it was reported that most of the bacterial diseases induces NF-κB related gene expression (Wang et al., 2017). Furthermore, (Verhelst et al., 2013; Kim et al., 2014) reported that IKBKKE was a major NF-κB signaling mediator. As the significant upregulation of most of NF-κB pathway genes (NFKBIA, NFKBIE and IKBKE) were detected, it seems that NF-κB pathway was activated in the chicken erythrocytes after M. synoviae infection. This study results suggests that NF-κB related genes may play key roles in regulating the immune response to bacterial infection in chickens.

The expression of NFKBIE gene was also significantly upregulated at certain time interval (2 h and 10 h) post infection in response to M. synoviae infected erythrocytes. This finding could be supported by biological knowledge since NF-κB signaling that is inhibited by NFKBIE is transcriptional factor involved in inflammatory immune response (Guthke et al., 2005). Moreover, it was also reported that NFKBIE itself is upregulated at mRNA level by TNF, whose over expression is feature of inflammatory diseases (Rao et al., 2010).

IFNs are cytokines produced in response to viral, bacterial, and fungal pathogens, as well as parasites. The effector mechanisms of IFN-α mainly derive from products of genes which are transcriptionally regulated by type I IFN signaling. Bacteria trigger type I IFN (IFN-α) production mostly following the recognition of bacterial nucleic acids or the Gram-negative cell wall component lipopolysaccharide (LPS) by innate immune receptors (Boxx and Cheng, 2016; Monroe et al., 2010). Our results revealed that IFN-α can be expressed in chicken erythrocytes in response to M. synoviae infection. Interestingly, we observed decreased expression level during early stage of infection (0 h, 2 h and 6 h) surprisingly; expression level increased significantly at later stage (10 h) post infection. It was recently found that chicken erythrocytes constitutively express transcripts for many TLRs as well as for some cytokines such as IFN-α, IFN-β, and IL-8 (Paolucci et al., 2013).

Our study result also reveals the significant up-regulation of MyD88 mRNA gene expression in chicken erythrocytes in response to M. synoviae strain. Previously it was reported that MyD88 gene constitutively expressed on almost all tissues (Hardiman et al., 1997). Likewise, it was shown previously that in spleen and thymus MyD88 gene was expressed higher, which is consistent with results of mouse and human (Hardiman et al., 1996, 1997). Previous studies also suggested an important role of MyD88 gene expression in NF-κB activation in-vitro in the chicken innate immune response to bacterial infections (Yafeng et al., 2008). Recently it was also stated that all TLR 3 employ the MyD88 dependent pathway (O’neil, 2006). The significant upregulation of MyD88 in erythrocyte suggested that this adaptor molecule plays an important role to induce an innate immune response to M. synoviae infection.

There is increasing evidence that MDA5 have additional distinct molecular functionalities in immune signaling (Kasumba and Grandvaux, 2018). It is well-established that the interferon regulatory factor (IRF) and innate immune NF-κB cytokine signaling pathways have many areas of cross-regulation and expression (Czerkies et al., 2018). Accordingly, MDA5 have been shown to activate NF-κB signaling during infection (Yoboua et al., 2010; Rückle et al., 2012). In current in-vitro study for first time it was reported that in M. synoviae infected chicken erythrocytes expression of MDA5 was upregulated in response to bacterial infection. Our results are in agreement with (Ye et al., 2018), their results showed up-regulated MDA5 expression after using a synthetic bacterial analog, Lipopolysaccharide (LPS), representing stimulation by Gram-negative bacteria. Several studies concurrently showed that LPS or bacterial challenge resulted in up-regulation of fish MDA5, such as common carp (Cyprinus carpio) challenged with Aeromonas hydrophila (Imaizumi et al., 2002). Furthermore, chicken MDA5 is ubiquitously expressed highest in intestine (Su et al., 2010; Huang et al., 2010). Hence, such results suggesting that MDA5 might not be involved exclusively in recognizing viral PAMPs, but they are also capable of indirectly distinguishing bacterial PAMPs chickens.

CCL5, a target gene of NF-κB activity, is expressed by T lymphocytes, macrophages, platelets, synovial fibroblasts, tubular epithelium, and certain types of tumor cells (Soria and Ben-Baruch, 2008). NF-κB activation by different stimuli such as CD40 (Aldinucci et al., 2012; or IL-15 Chenoweth et al., 2012) induces CCL5 production. In current in-vitro study, infection of erythrocytes with M. synoviae in chicken significantly upregulated the mRNA expression of CCL5. Previously upregulated expression of CCL5 was reported by (Majumder and Siibart, 2015) in chicken macrophages upon direct contact of HD-11 cells with M. gallisepticum. In addition, in 4T1 tumour cells CCL5 was constitutively expressed with upregulated expression (Kurt et al., 2001), suggesting that in resistance to immune-surveillance high levels of CCL5 may play an important role (Adler et al., 2003). Moreover, higher expression of CCL5 gene maybe due to its transcriptional activation that critically dependent on NF-κB signaling pathway (Dandan et al., 2013).

TRAF6 has been linked to the function of various immune effector cells. Previous studies have shown that TRAF6 regulates several signaling cascades involved in
adaptive immunity and innate immunity (Ye et al., 2002). TRAF6 recognizes different binding sites of CD40 and receptor activator of nuclear factor κB, and other members of the TNFR superfamily (Pullen et al., 1998). In addition, MDA5 signaling pathway requires the mitochondrial protein ISP-1 to form a signaling complex with TRAF6, which leads to the activation of TRAF6 and initiation of the innate immune response (West et al., 2011). In our study the expression of TRAF6 was significantly upregulated in response to M. synoviae infected erythrocytes, suggesting that TRAF6 is essential for the induction of effective innate immune responses in chicken erythrocytes. Previously it was observed that abundant TRAF6 expression was in the spleen, largest lymph organ, in mice, ducks and humans, suggesting that TRAF6 could be a vital factor for the immune system (Jin et al., 2017). In addition, (Stockhammer et al., 2010) revealed that in immune-related tissue, such as thymus, spleen and bursa of Fabricius the mRNA expression of TRAF6 was highest, indicated an innate immune response mediated by TRAF6 in chickens to pathogenic challenges. Hence, TRAF6 expression indicated that chicken erythrocytes might have an important role in chicken defense against bacterial infections.

Lastly, in the current study, we examined the mRNA gene expression of cMGF in infected chicken erythrocytes. Our results revealed for the first time that M. synoviae infected chicken erythrocytes, there is down regulation of cMGF gene in the early phase such as 0h and 2h post infection while with passage of time expression enhanced significantly in later time intervals when compared with an uninfected group. cMGF is a 27–kDa glycoprotein that was first described regarding its capability to encourage the growth of macrophage and granulocyte colonies from avian bone marrow progenitor cells in vitro (Leutz et al., 1984). Previously it was reported the involvement of cMGF in the innate immune response through enhancing monocyte or macrophages quantity and activation, resulting in the production of NO with antiviral activity (Djeraba et al., 2002). Furthermore, it was also declared that cMGF is required for survival and growth for normal and transformed avian myelomonocytic cells (Leutz et al., 1984, 1988). Together, all these results show that chicken erythrocytes may have the potential to respond to bacterial infectious agents.

CONCLUSION

This in vitro study provided the first evidence that M. synoviae interacts with chicken erythrocytes which can also constitutively express NF-κB signaling and other immune system genes. Expression of most of NF-κB signaling pathway and other immune system genes were significantly regulated in chicken erythrocytes after M. synoviae infection, indicating their involvement in immune response to bacterial infection. Future studies may be planned to understand how NF-κB signaling and other immune system genes respond to bacterial infections in chicken erythrocytes and their roles in the host’s immunity.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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