European Journal of Inflammation

Original Research Article

Different innate immunity and clearance of *Salmonella Pullorum* in macrophages from White Leghorn and Tibetan Chickens

European Journal of Inflammation
Volume 16: 1–9

DOI: 10.1177/2058739218780039
journals.sagepub.com/home/eji
SAGE

Shao Wei,[1,2] Keliang Wu,[1,2] Yijuan Nie,[1,2] Xiang Li,[1] Zhengxing Lian[1,2] and Hongbing Han[1,2]

Abstract

Salmonella enterica serovar Gallinarum biovar Pullorum (*S. Pullorum*) is responsible for the systemic salmonellosis in different breeds of chickens. Macrophages, as host cells, play a key role in the innate immune response following infection with *S. Pullorum*. In this study, we first generated macrophages from two breeds of chicken (White Leghorn (WL) and Tibetan Chickens (TC)) peripheral blood monocytes in vitro. Then, we showed that the production of interleukin-1β (IL-1β), macrophage inflammatory protein-1β (MIP-1β) and interleukin-10 (IL-10) in lipopolysaccharide (LPS)-treated macrophages was significantly higher compared with the unstimulated cells in TC. LPS triggered only more expression of IL-10 in WL macrophages. Furthermore, macrophages from TC eliminated intracellular bacteria more efficiently than those from WL after *S. Pullorum* infection at a multiplicity of infection (MOI) 1. In addition, the variation between individuals and sex had the crucial effect on the immune response to LPS and *S. Pullorum* invasion.

Keywords

immune response, macrophages, *Salmonella Pullorum*, Tibetan Chickens, White Leghorn

Date received: 27 February 2018; accepted: 8 May 2018

Introduction

Salmonella Pullorum is a kind of common poultry pathogen which causes severe systemic infection leading to a high mortality of young birds. A range of symptoms such as the decrease in egg-laying rate, the loss of weight, and the infection of reproductive tract result in vertical transmission to progeny.[1,2] Pullorum disease had been frequently reported in developing countries.[3] The measures to control *S. Pullorum* disease are based on the use of vaccines and antibiotics or the detection and slaughter of infected breeder flocks. However, the massive use of vaccines and antibiotics in poultry results in residues of chemicals in meat food, which is harmful to our health and enhances strains antibiotic resistance. Moreover, the persistent infection in convalescent chickens is undetectable. Therefore, the breeding of chickens with strong natural resistance to bacteria is an effective strategy.

Macrophages were important effector cells in the progression of avian systemic infection.[4] Those cells are equipped with numbers of pathogen-recognition receptors that make them respond to bacterial

[1]Beijing Key Laboratory for Animal Genetic Improvement, College of Animal Science and Technology, China Agricultural University, Beijing, China
[2]National Engineering Laboratory for Animal Breeding, College of Animal Science and Technology, China Agricultural University, Beijing, China

Corresponding author:
Hongbing Han, Beijing Key Laboratory for Animal Genetic Improvement, College of Animal Science and Technology, China Agricultural University, NO.2 Yuan Ming Yuan West Road, Haidian District, Beijing 100193, P.R. China.
Email: hanhongbing@cau.edu.cn

infection and induce the massive production of inflammatory cytokines, oxygen-dependent antimicrobial reactive oxygen species (ROS), and reactive nitrogen species including H_2O_2 and NO to kill intracellular *Salmonella*.[5–8] Although the mechanism of *S. Pullorum* persisting in chickens was still unrevealed, the role of macrophages was obviously recognized in persistent infection through pathogenicity island 2 type III secretion system.[9] Researchers also suggested that macrophages from *Salmonella*-resistant chickens, expressing more pro-inflammatory cytokines and chemokines, were more efficiently in clearing bacteria than cells from susceptible chickens in vitro.[10,11] In addition, Williams et al.[12] indicated that there were different outcomes in fast- and slow-growing broiler chickens after *Campylobacter jejuni* infection.

During the 1970s, definitive evidence revealed that macrophages were a crucial cell type in the recognition of lipopolysaccharide (LPS).[13] LPS, as an endotoxin, exists in the cell outer membrane of gram-negative bacteria. In vitro, it could mimic gram-negative bacteria to initiate cellular immune response and induce chicken cells increased messenger RNA (mRNA) levels of pro-inflammatory cytokines, which targets to adaptive immune responses.[14]

The aim of this study was to assess the differences of innate immunity to LPS and clearance of *S. Pullorum* in macrophages from White Leghorn (WL) and Tibetan Chickens (TC), which provided a new strategy for the breeding of resistant chicken.

Materials and methods

Ethics statement

All experimental animal protocols were approved by the Animal Care and Use Committee at China Agricultural University (approval ID 2013–011). All samples were obtained from the wing vein of chickens.

Experimental animals and strain

In total, 10 WL (5 males, 5 females), 11 TC (5 males, 6 females), aged 26 weeks, were raised under an temperature of 20°C and fed with food and water ad libitum at Experimental Chicken Farm of China Agricultural University. The C79-13 strain *S. Pullorum* was purchased from China Institute of Veterinary Drugs Control.

Reagents

The primary antibodies CD14 (bs-1192R), MIP-1β (CCL4, bs-2475R), and MCSFR (bs-2755R) were purchased from Beijing Biosynthesis Biotechnology. Anti-iNOS antibody (ab3523) and Anti-*Salmonella* antibody (ab35156) were purchased from Abcam. KUL01 (8420-09) was purchased from SouthernBiotech. The Alexa Fluor® 594 conjugate goat anti-rabbit secondary antibody (A11012) was purchased from Life Technologies.

Cell collection and culture

The monocytes were collected as described as previously.[15] Briefly, heparinised blood samples were taken from the wing vein of chickens and then diluted 1:1 with Hanks' buffer. Subsequently, the suspension was added slowly to the equivalent volume of lymphocytes separation medium (Tianjin HaoYang Biotech, Inc., China) and then centrifuged for 20 min at 400–500 g. The middle layer was transferred to a new tube and washed three times in Hanks' buffer. The cells were resuspended with RPMI1640 (Gibco Life Technologies) supplemented with 10% fetal bovine serum (Gibco Life Technologies), 100 U/mL penicillin, 50 μg/mL streptomycin, and 0.1 mM 2-mercaptoethanol (Sigma). Cells (1×10^6/mL) were seeded to different plates. Nonadherent cells were removed with changes in medium after 48 h. Cells were assayed at 7 days.

Phagocytosis assay

Cells were seeded into 48-well plates and co-incubated with FITC-latex beads (Sigma) at a ratio of 5:1 bead to cell ratio for 20 h at 37°C, 5% CO_2. Cells were washed with phosphate-buffered saline (PBS) and analyzed with ImageXpress Micro (Molecular Devices, USA).

Immunofluorescence assay

Cells were fixed with 4% paraformaldehyde for 20 min at room temperature (RT). Then, cells were permeabilized with 0.3% TritonX-100 for 15 min and blocked in blocking solution for 1 h at RT. Then, cells were incubated with primary antibodies at 4°C overnight. Cells were washed in PBS containing 0.3% TritonX-100 and incubated the secondary antibodies for 1 h at RT. After that, nuclei were stained with DAPI (Sigma). In addition, cells

Table 1. All primer sequences used in the study.

Gene	Forward sequence (5′–3′)	Reverse sequence (5′–3′)
TLR4	CCAAACACCACCCTGGACTT	TGTATGGATGTGGCACCTTGA
CD14	GGGACCTGGAGAAATACGCTG	GCAGTGACACAATGGGCAAG
CD80	ACCGAGTGTATTGGCAGATG	ACAGGCTCTCCACACAAAAG
CD200R1	AGCCATCATTCTCAGTTTCCT	ACCTCCACTGTGTCATCCTG
MRC2	CTGCCCAAAGGAACTGATGT	CTGCCATCGTTCTCATAGGTG
TREM	CACCACTTCCATCAGCGACA	TCAGCACAGCCACCACAAAC
CD36	TACTGGGAAGGTTACTGCGA	TCACGGTCTTACTGGTCTGG
SCARA3	ACAACATCTCCTCCTTCCTG	CGTTGATGTTGGTGAAGATG
MARCO	AGCAAGGGTGATTATGGCAG	GGTTGTCCCAGTCATCATCAC
MHC II	CATCTACAACCGGCAGCAGTT	CGCCGAGACCCTCACCTT
iNOS	GGTAACAGCGGAAGGAGAC	GTGTCATGTCACAGGAGGAAC
Arginase2	ATGGGCAGGAGACAGAGAC	CCTGTGTTGTGTATTTCCTCTG
MIP-1β	CTCCTCATTGCCATCTGCTAC	CTGGCTGTTGGTCTCGTAGTAG
IL-8	AGGATGGAAGAGAGGTGTGCT	CGATGTGAAAGGTGGAAGATG
IL-1β	AGAAGAAGCCTCGCCTGGATT	GACGGGCTCAAAAACCTCCTC
TNF-α	CCGCCCAGTTCAGATGAGTTG	AGAGCATCAACGCAAAAGGGA
IL-10	CAATCCAGGGACGATGAACTT	AGGTGAAGAAGCGGTGACAG

were incubated with Mouse Anti-Chicken Monocyte/Macrophage-PE KUL01 for 1 h at 37°C and washed with PBS.

Cell treatment

Cells were stimulated with 1 μg/mL LPS of *Escherichia coli* 0111: B4 (Sigma) after 7 days of culture. In the infection experiment, *S. Pullorum* (C79-13 strain) was overnight cultured in Luria-Bertani (LB) broth at 37°C. Cells were infected with *Salmonella* at a 1:10 multiplicity of infection (MOI). After 30 min incubation, cells were washed twice. Then, RPMI-1640 supplemented with gentamicin (100 μg/mL) was added to cells for 1 h to kill extracelluar bacteria and then replaced with less gentamicin (20 μg/mL). The infection rate of cells was counted by MetaMorph after 0.5 h. Meanwhile, the bacteria were harvested by lysis of cells with PBS containing 0.3% TritonX-100, and the number of intracelluar survival bacteria at 0.5, 12, and 24 h was counted on LB agar.[10]

Real-time quantitative polymerase chain reaction amplifications

RNA was extracted using RNeasy Plus Mini Kit (Qiagen) according to the manufacturer's instructions. The first complementary DNA (cDNA) synthesis was performed referring to RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific). Polymerase chain reaction (PCR) amplifications were carried out following the conditions: one cycle of 95°C for 5 min at the first stage, 35 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 15 s at the step 2, 72°C for 7 min at the final stage. The primers used in the study are showed in Table 1. The relative cytokine expressions were calculated with $2^{-\Delta\Delta CT}$ method. In Figure 2, $\Delta\Delta C_T = ((C_{T,Target} - C_{T,GAPDH})_{3h} - (C_{T,Target} - C_{T,GAPDH})_{0h})$. In Figure 3, to show the various cytokine expressions of macrophages between individuals from the same breed in the absence or presence of LPS, we first calculated the $(C_{T,Target} - C_{T,GAPDH})_{untreated}$ of every chick and got the average value "ΔC_T." Then, the relative cytokine expressions of LPS-treated and LPS-untreated macrophages from different individuals were, respectively, calculated with $2^{-((CT,Target\text{-}CT,GAPDH)\ untreated\ -\ \Delta CT)}$ or $2^{-((CT,Target\ -\ CT,GAPDH)3h\ -\ \Delta CT)}$.

Statistical analysis

All data were analyzed by SPSS software. T-test was performed to determine *P* value. The level of significance was defined as $P < 0.05$. MD ImageXpress Micro was used for the statistics of the proportion of positive cell in immunofluorescence and the number of intracellular bacteria.

Results

The identification of peripheral blood monocytes–derived macrophages in vitro

Monocytes were isolated from peripheral blood of WL selected randomly. After 7 days, cells became

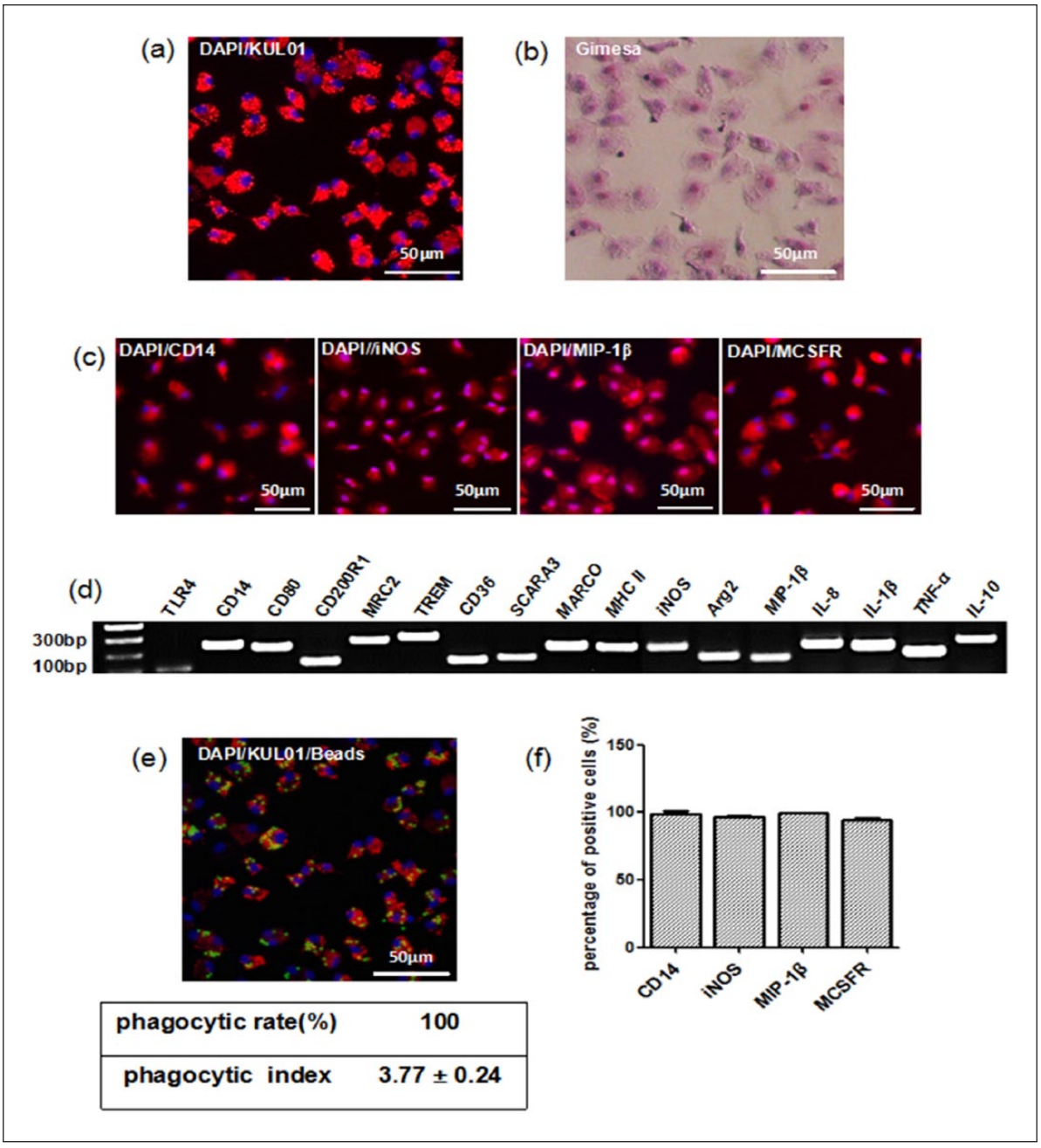

Figure 1. Peripheral blood monocytes differentiate into macrophages in vitro. (a) Immunofluorescence of KUL01 in monocyte/macrophage cultured after 7 days in vitro. KUL01, red signal; DAPI, blue signal. (b) Giemsa staining showed the morphology of macrophages with round nuclei unlike monocytes with horseshoe-shaped nuclei. (c) Immunofluorescence expression of CD14, iNOS, MIP-1β, and MCSFR (red) in macrophages from female chickens after 7-day culture. DAPI (blue). (d) PCR was carried out to detected *TLR4, CD14, CD80, CD200R1, MRC2, TREM, CD36, SCARA3, MARCO, MHCII, iNOS, Arginase2, MIP-1β, IL-8, IL-1β, TNF-α*, and *IL-10*. (e) Immunofluorescence showed phagocytic capacity of cells. KUL01 (red), beads (green), DAPI (blue). (f) Statistical analysis of CD14+, iNOS+, MIP-1β+, and MCSFR+ cells in positive rate.

large and showed tight adhesion in vitro. To further identify the cells, we assessed the expression of KUL01, marker of the mononuclear phagocyte system that consists of chicken monocytes, macrophages, and interdigitating cells.[16] KUL01+ (Figure 1(a)) and a typical macrophage morphology (Figure 1(b)) were found in cells, respectively. Moreover, the proteins including CD14, inducible nitric oxide synthase (iNOS), MIP-1β, and macrophage colony-stimulating factor receptor (MCSFR) were stained by immunofluorescence (Figure 1(c)–(f)). Other genes expressed by macrophages were

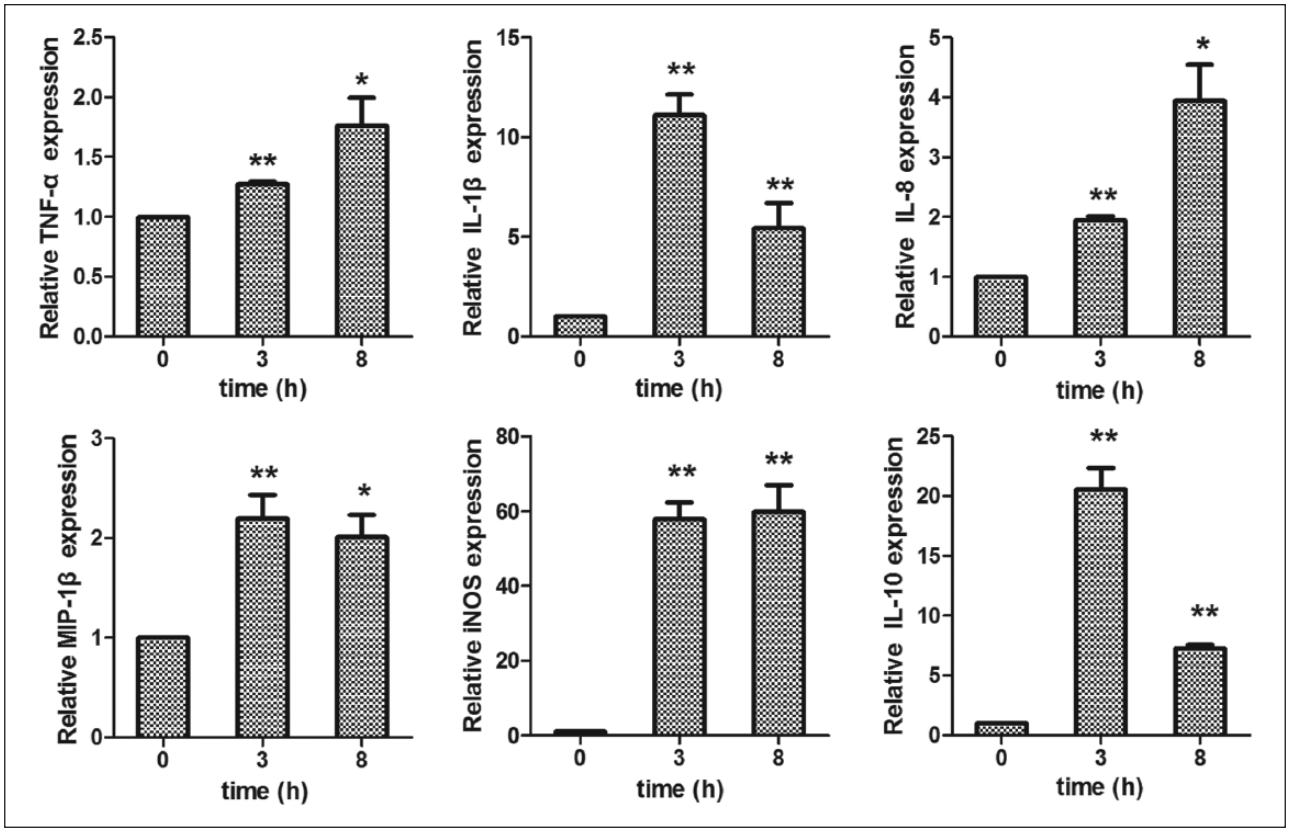

Figure 2. Relative inflammatory cytokines expressions in 1 μg/mL LPS-stimulated cells derived from peripheral blood monocytes at 3 and 8 h. *$P < 0.05$, **$P < 0.01$. *3 and 8 h versus 0 h, respectively.

also detected (Figure 1(d)) by PCR. The study also indicated that these cells were efficient at phagocytosis with the phagocytic rate of 100% and phagocytic index of 3.77 ± 0.24 (Figure 1(e)). In addition, LPS elicited the expression levels of inflammatory cytokines *TNF-α, IL-1β, IL-8, MIP-1β, IL-10*, and *iNOS* at 3 and 8 h (Figure 2). These results indicated that monocytes-derived macrophages can be prepared from peripheral blood.

LPS elicits stronger innate immune responses in macrophages from TC

To identify the differences of innate immune response to LPS in macrophages from WL and TC, we measured the mRNA levels of inflammatory cytokines in cells following 3 h incubation with 1 μg/mL LPS. LPS stimulated higher release of *IL-1β* ($P < 0.01$), *MIP-1β* ($P < 0.05$), and *IL-10* ($P < 0.01$) of macrophages compared with the unstimulated cells in TC (Figure 3(a)). We also demonstrated that LPS-treated WL macrophages released significantly higher of IL-10 ($P < 0.05$) than LPS-untreated cells (Figure 3(a)), which can contribute to the male (Figure 3(b)). Moreover, macrophages from female showed stronger expression of *MIP-1β* ($P < 0.05$), *iNOS* ($P < 0.05$), and *IL-10* ($P < 0.01$) in TC (Figure 3(c)) at 3 h after LPS stimulation. Considerable differences could also be found in the response to LPS stimulation between individuals within one breed due to genetic differences. Together, these results suggested that LPS induced higher immunity in TC macrophages.

More effective clearance of S. Pullorum *in TC macrophages*

Macrophages from WL and TC were infected with *S. Pullorum* in vitro at an MOI of 1 and a high level MOI of 10. The percentage of *S. Pullorum*–infected cells was higher at an MOI of 10 at 0.5 h postinfection (p.i.) (Figure 4(a)), while no significant differences were seen between two breeds at any MOI (Figure 4(b)). The numbers of intracellular bacteria was strongly decreased in cells from two breeds infected for an additional 12 and 24 h at an MOI of 1 (Figure 4(c)) and an MOI of 10 (data not shown) with the exception of the cells from two WL

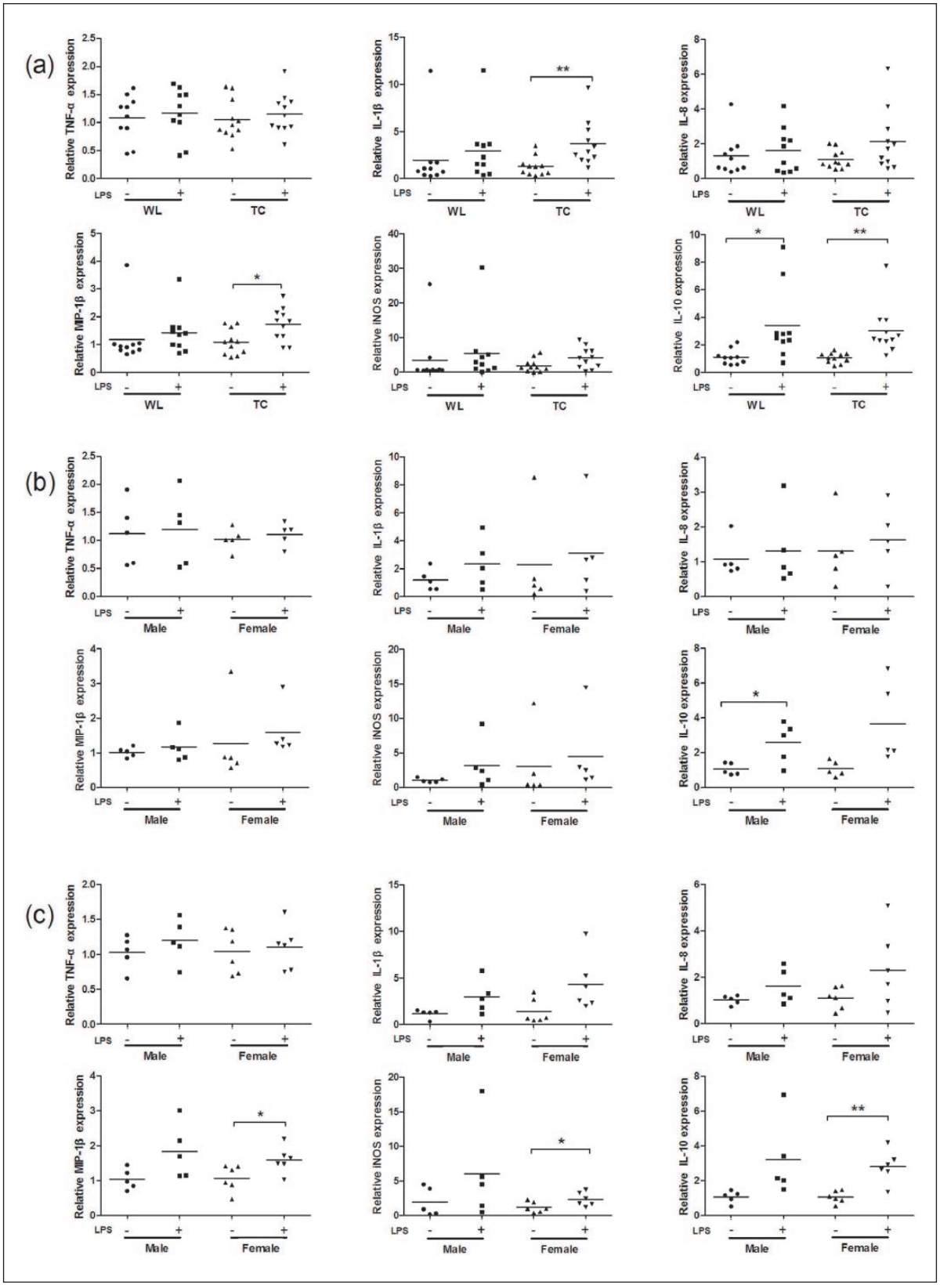

Figure 3. The effect of LPS on the mRNA level of cytokine, chemokine, and enzyme . (a) Fold changes of genes expressions in macrophages from White Leghorn (WL) and Tibetan Chickens (TC) at 3 h in the presence or absence of LPS (1 μg/mL). *$P < 0.05$, **$P < 0.01$. (b and c) The analysis of genes expressions in macrophages from male and female at the same breed. (b) WL; (c) TC. *$P < 0.05$. Each symbol represents the value for an individual bird. The black bar represents the median value for each group.*LPS-stimulated cells versus LPS-unstimulated cells.

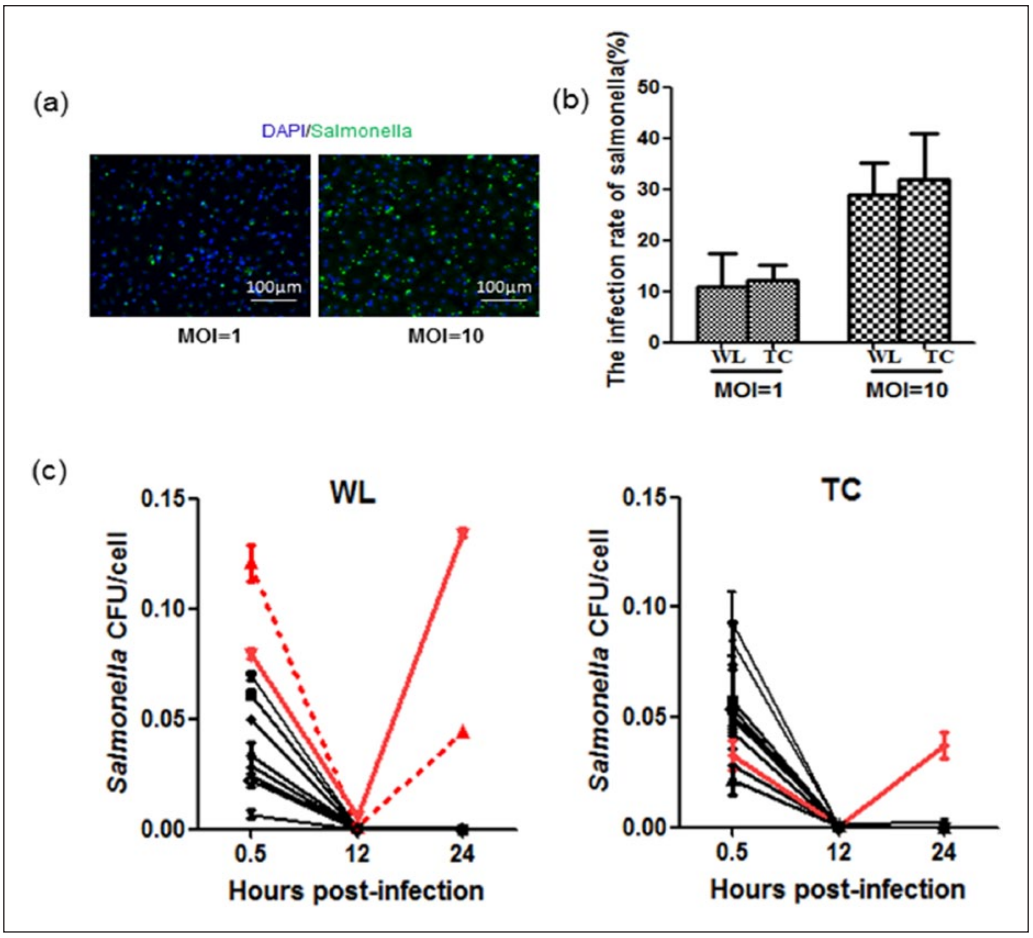

Figure 4. Survival of *Salmonella* within macrophages from WL and TC. (a) Immunofluorescence for *Salmonella Pullorum* within macrophage at an MOI 1 and 10 at 0.5 h post-challenge. DAPI (blue), bacteria (green). (b) Analysis of macrophage infection rate with *S. Pullorum* at MOI 1, 10 after 0.5 h post-infection. (c) The number of intracellular bacteria in macrophages at MOI 1 for every individual at 0.5, 12, and 24 h after *S. Pullorum* infection. The solid and dotted redlines represented those birds whose intracellular bacterial loads were significantly increased compared to others at 24 h post-infection. (Left, WL; right, TC).

(female) and one TC (male) whose bacterial loads increased at 24 h p.i. at an MOI of 1. The data above suggested that macrophages from TC could eliminate intracellular *S. Pullorum* more efficiently than WL and cells from different individuals chicken within breeds showed considerable abilities to control bacterial infection at a low MOI.

Discussion

Macrophages have been considered to be significant immune effector cells and the major models to study the infection and clearance of *Salmonella* in vitro. In this study, we obtained KUL01+ cells, marker of the monocytes and macrophages from chicken, which demonstrated the high purity of monocytes from peripheral blood (Figure 1(a)). Macrophages recognized endogenous antigen through pathogen-recognition receptors that made them participate in phagocytosis and secrete inflammatory cytokines.[5] Our study also indicated that cells derived from peripheral blood co-cultured with microbeads were efficient at phagocytosis, which showed differentiation of monocytes into macrophages (Figure 1(e)). Moreover, LPS drove the induction of cytokines *TNF-α, IL-1β, IL-8, MIP-1β, IL-10*, and *iNOS* (Figure 2) in cells. So, we confirmed that macrophages could arise from the differentiation of peripheral blood monocytes.

It had been considered that the innate immune response could propel the antigens to elicit the acquired immunity.[14,17,18] However, pathogens including *S.* Typhimurium and Mycobacteria could delay acquired immune response.[19,20] Thus, innate immune responses performed the initial immunological function of

resistance to such pathogens. Previous findings revealed that macrophages from the *Salmonella*-resistant chickens presented rapid and strong pro-inflammatory cytokines release after being challenged with *Salmonella.*[11] As the major component of gram-negative bacteria, LPS recognition was the crucial initiators of the early innate immune response. Then, we made efforts to compare LPS-induced production of cytokines in macrophages isolated from WL and TC, respectively. Results showed that macrophages from TC produced higher level of pro-inflammatory *IL-1*β ($P < 0.01$), chemokines *MIP-1β/CCL4* ($P < 0.05$) which mediated host defense and pathological inflammatory responses by recognizing CCR5 in the presence of LPS compared with no LPS.[21] The significantly increased production of *IL-10* ($P < 0.01$) indicated the ability of regulating inflammatory response in TC cells (Figure 3(a)). In addition, significantly higher expressions of chemokines *MIP-1β* ($P < 0.05$), *iNOS* ($P < 0.05$), and anti-inflammatory cytokine *IL-10* ($P < 0.01$) were also detected in macrophages from female than those from male in TC (Figure 3(c)). This result suggested that macrophages from female had access to generate immunity to LPS and were more effective to regulate the balance of inflammatory response.

It had been demonstrated that the most of bacteria would be eliminated by 24 h post-challenge in the resistant macrophages.[10] Consistent with that, macrophages from the majority of two breeds presented high level in clearing intracellular bacteria at 24 h of MOI 1 and 10. Interestingly, we found macrophages from two WL and one TC could not clear intracellular *Salmonella* efficiently at an MOI of 1, which demonstrated that macrophages from TC were more effective at the clearance of intracellular *S. Pullorum* than those from WL. It has been suggested that the capacity of *Salmonella* to proliferate within macrophages relied on the individual genetic contribution of macrophages.[22,23] Previous study also suggested differences appeared between individuals within groups in the response to *C. jejuni* infection.[24] In our study, considerably different abilities to eliminate bacteria in different individuals' macrophages within breeds were seen due to genetic differences between individuals, which would be of great values to the exploration on the pathogenesis of *S. Pullorum* (Figure 4(c)).

Acknowledgements

S.W. and K.W. contributed equally to this study.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

This work was supported by National Key R&D Program (2016YFD0501200).

References

1. Wigley P, Berchieri A Jr, Page KL et al. (2001) Salmonella enterica serovar Pullorum persists in splenic macrophages and in the reproductive tract during persistent, disease-free carriage in chickens. *Infection and Immunity* 69(12): 7873–7879.
2. Wigley P, Hulme SD, Powers C et al. (2005) Infection of the reproductive tract and eggs with Salmonella enterica serovar Pullorum in the chicken is associated with suppression of cellular immunity at sexual maturity. *Infection and Immunity* 73(5): 2986–2990.
3. Barrow PA and Freitas Neto OC (2011) Pullorum disease and fowl typhoid—new thoughts on old diseases: A review. *Avian Pathology* 40(1): 1–13.
4. Chappell L, Kaiser P, Barrow P et al. (2009) Theimmunobiology of avian systemic salmonellosis. *Veterinary Immunology and Immunopathology*128(1–3): 53–59.
5. Gordon S (2002) Pattern recognition receptors: Doubling up for the innate immune response. *Cell* 111(7): 927–930.
6. Bogdan C, Röllinghoff M and Diefenbach A (2000) Reactive oxygen and reactive nitrogen intermediates in innate and specific immunity. *Current Opinion in Immunology* 12(1): 64–76.
7. Fang FC (2004) Antimicrobial reactive oxygen and nitrogen species: Concepts and controversies. *Nature Reviews Microbiology* 2(10): 820–832.
8. He H, Genovese KJ, Swaggerty CL et al. (2012) A comparative study on invasion, survival, modulation of oxidative burst, and nitric oxide responses of macrophages (HD11), and systemic infection in chickens by prevalent poultry Salmonella serovars. *Foodborne Pathogens and Disease* 9(12): 1104–1110.
9. Wigley P, Jones MA and Barrow PA (2002) Salmonella enterica serovar Pullorum requires the Salmonella pathogenicity island 2 type III secretion system for virulence and carriage in the chicken. *Avian Pathology* 31(5): 501–506.
10. Wigley P, Hulme SD, Bumstead N et al. (2002) In vivo and in vitro studies of genetic resistance to systemic salmonellosis in the chicken encoded by the SAL1 locus. *Microbes and Infection* 4(11): 1111–1120.
11. Wigley P, Hulme S, Rothwell L et al. (2006) Macrophages isolated from chickens genetically resistant or susceptible to systemic salmonellosis show magnitudinal

and temporal differential expression of cytokines and chemokines following Salmonella enterica challenge. *Infection and Immunity* 74(2): 1425–1430.
12. Williams LK, Sait LC, Trantham EK et al. (2013) Campylobacter infection has different outcomes in fast- and slow-growing broiler chickens. *Avian Diseases* 57(2): 238–241.
13. Moore RN, Goodrum KJ and Berry LJ (1976) Mediation of an endotoxic effect by macrophages. *Journal of the Reticuloendothelial Society* 19(3): 187–197.
14. Ciraci C, Tuggle CK, Wannemuehler MJ et al. (2010) Unique genome-wide transcriptome profiles of chicken macrophages exposed to Salmonella-derived endotoxin. *BMC Genomics* 11: 545.
15. Sun SF, Pan QZ, Hui X et al. (2008) Stronger in vitro phagocytosis by monocytes-macrophages is indicative of greater pathogen clearance and antibody levels in vivo. *Poultry Science* 87(9): 1725–1733.
16. Mast J, Goddeeris BM, Peeters K et al. (1998) Characterisation of chicken monocytes, macrophages and interdigitating cells by the monoclonal antibody KUL01. *Veterinary Immunology and Immunopathology* 61(2–4): 343–357.
17. Bendelac A and Fearon DT (1997) Innate pathways that control acquired immunity. *Current Opinion in Immunology* 9(1): 1–3.
18. Janeway CA Jr and Medzhitov R (2002) Innate immune recognition. *Annual Review of Immunology* 20(1): 197–216.
19. Luu RA, Gurnani K, Dudani R et al. (2006) Delayed expansion and contraction of CD8+ T cell response during infection with virulent Salmonella typhimurium. *Journal of Immunology* 177(3): 1516–1525.
20. Albaghdadi H, Robinson N, Finlay B et al. (2009) Selectively reduced intracellular proliferation of Salmonella enterica serovar typhimurium within APCs limits antigen presentation and development of a rapid CD8 T cell response. *Journal of Immunology* 183(6): 3778–3787.
21. Cheung R, Malik M, Ravyn V et al. (2009) An arrestin-dependent multi-kinase signaling complex mediates MIP-1beta/CCL4 signaling and chemotaxis of primary human macrophages. *Journal of Leukocyte Biology* 86(4): 833–845.
22. Schwan WR, Huang XZ, Hu L et al. (2000) Differential bacterial survival, replication, and apoptosis-inducing ability of Salmonella serovars within human and murine macrophages. *Infection and Immunity* 68(3): 1005–1013.
23. Raupach B and Kaufmann SH (2001) Bacterial virulence, proinflammatory cytokines and host immunity: How to choose the appropriate Salmonella vaccine strain? *Microbes and Infection* 3(14–15): 1261–1269.
24. Humphrey S, Chaloner G, Kemmett K et al. (2014) Campylobacter jejuni is not merely a commensal in commercial broiler chickens and affects bird welfare. *MBio* 5(4): e1364.