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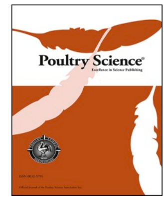

Chicken NLRP3 monoclonal antibody development and its antigenic epitope identification

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ABSTRACT

Inflammation is key important pathogenic response that occurs in several poultry diseases. NOD-like receptor protein 3 inflammasome plays a central role in initiating inflammation. However, the lack of antibodies against chicken-derived inflammatory factors presents a major bottleneck in related studies. In this study, we developed a monoclonal antibody (mAb) against chicken NLRP3 using hybridoma technology. This antibody can recognize both eukaryotic and prokaryotic NLRP3 proteins as well as the upregulation of endogenous NLRP3 in HD11 cells induced by lipopolysaccharide or infectious bursal disease virus. Furthermore, a novel antigenic epitope, 42DELEKVTHPSS52, located in the PYD domain of chicken NLRP3 and specifically recognized by this mAb was identified. This epitope is unique to chickens and valuable for distinguishing chicken NLRP3 from orthologs in other species. The developed mAb provides an important tool for detecting chicken NLRP3 and facilitates further study of its antigenic structure and biochemical characteristics.

Introduction

The inflammatory response is a host immune response triggered by pathogenic microorganisms or other stimuli. Inflammasomes play a central role in the inflammation-triggering process, with the NOD-like receptor protein 3 (NLRP3) inflammasome being the most widely studied to date. NLRP3 comprises three domains: PYD, NACHT, and LRR. When host cells sense danger signals through pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs), NLRP3 undergoes oligomerization via homotypic interactions between NACHT domains (Fu J, et al., 2023). The apoptosis-associated speck-like protein containing CARD (ASC) is recruited by NLRP3 through the PYD domain (Yang et al., 2019). ASC interacts with the CARD domain of pro-caspase-1 to assemble NLRP3 inflammasomes to produce activated caspase-1, which triggers subsequent signal transduction and inflammatory responses (Elliott, et al., 2015). Therefore, NLRP3 is a biomarker of the inflammatory response.

Poultry farms are susceptible to various diseases. The inflammatory response is an important pathogenic process in many poultry diseases (Fusco, et al., 2020; Chen, et al., 2023; Zhang, et al., 2023). However, the molecular mechanisms by which avian pathogens trigger inflammation remain unclear, and the lack of antibodies for chicken-derived inflammatory factors is an important bottleneck in related research (Liu, et al., 2019). In this study, we prepared a monoclonal antibody (mAb) targeting chicken NLRP3 and identified, for the first time, an antigenic epitope in the PYD domain of chicken NLRP3. This study has important value for in-depth research on the inflammatory mechanisms of poultry diseases.

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Table 1
Primers.

Primer name	Primer sequence (5′–3′)
PYD-F	CATCATTTTGGCAAAGAATTCGCCACCATGGTGAGCAAGGGCGAG
PYD-R	GAAAAAGATCTGCTAGCTCGAGTCAATCAAGGATTTTTTCAGCAA
NACHT-F	CATCATTTTGGCAAAGAATTCGCCACCATGGTGAGCAAGGGCGAG
NACHT-R	AAAAAGATCTGCTAGCTCGAGTCAGTCATTCCCAAAATACCTGTGG
LRR-F	CATCATTTTGGCAAAGAATTCGCCACCATGGTGAGCAAGGGCGAG
LRR-R	GAAAAAGATCTGCTAGCTCGAGTCACCGCAGGACACGCAGCTGG
P1-F	GCATGGACGAGCTGTACAAGAGCACCATCCTCTTAGAAGC
P1-R	GAAAAAGATCTGCTAGCTCGAGTCACTTCTTGAACTCTTG
P2-F	GCATGGACGAGCTGTACAAGTTCCAAGAGTTCAAGAAGAA
P2-R	GAAAAAGATCTGCTAGCTCGAGTCAGACCTTCTCCAGCTCATCCC
P3-F	GCATGGACGAGCTGTACAAGGATGAGCTGGAGAAGGTCAC
P3-R	GAAAAAGATCTGCTAGCTCGAGTCAGTCCATAGCAGCACCTTCCC
P4-F	GCATGGACGAGCTGTACAAGGAAGGTGCTGCTATGGACATTGCCA
P4-R	AAAAGATCTGCTAGCTCGAGTCAATCAAGGATTTTTTCAGCAAGGT
L1-F	CATCATTTTGGCAAAGAATTCGCCACCATGGTGAGCAAGGGCGAG
L1-R	GAAAAAGATCTGCTAGCTCGAGTCAAGCAGCACCTTCCCCATAGC
L2-F	CATCATTTTGGCAAAGAATTCGCCACCATGGTGAGCAAGGGCGAG
L2-R	AAAGATCTGCTAGCTCGAGTCAACCTTCCCCATAGCTGTC
L3-F	CATCATTTTGGCAAAGAATTCGCCACCATGGTGAGCAAGGGCGAG
L3-R	AGATCTGCTAGCTCGAGTCACCCATAGCTGTCGCCCATAT
R1-F	CATCATTTTGGCAAAGAATTCGCCACCATGGTGAGCAAGGGCGAG
R1-R	TGCTAGCTCGAGTCAGTCCATAGCAGCACCTTCCCCATAGCTGTCGCCCATATAACTGATGAGTGAGGAAGGATGGGTGACCTTCTCCAGCTTGTACAGCTCGTCCAT
R2-F	CATCATTTTGGCAAAGAATTCGCCACCATGGTGAGCAAGGGCGAG
R2-R	AGATCTGCTAGCTCGAGTCAGTCCATAGCAGCACCTTCCCCATAGCTGTCGCCCATATAACTGATGAGTGAGGAAGGATGGGTGACCTTCTTGTACAGCTCGTCCAT
R3-F	CATCATTTTGGCAAAGAATTCGCCACCATGGTGAGCAAGGGCGAG
R3-R	CTGCTAGCTCGAGTCAGTCCATAGCAGCACCTTCCCCATAGCTGTCCATATAACTGATGAGTGAGGAAGGATGGGTCTTGTACAGCTCGTCC
L4-F	CATCATTTTGGCAAAGAATTCGCCACCATGGTGAGCAAGGGCGAG
L4-R	GATCTGCTAGCTCGAGTCAGTCGCCCATATAACTGATGAGTGAGGAAGGATGGGTGACCTTCTCCAGCTCATCCTTGTACAGCTCGTCCAT
L5-F	CATCATTTTGGCAAAGAATTCGCCACCATGGTGAGCAAGGGCGAG
L5-R	AGATCTGCTAGCTCGAGTCAATAACTGATGAGTGAGGAAGGATGGGTGACCTTCTCCAGCTCATCCTTGTACAGCTCGTCCAT
L6-F	CATCATTTTGGCAAAGAATTCGCCACCATGGTGAGCAAGGGCGAG
L6-R	AGATCTGCTAGCTCGAGTCAGAGTGAGGAAGGATGGGTGACCTTCTCCAGCTCATCCTTGTACAGCTCGTCCAT
L7-F	CATCATTTTGGCAAAGAATTCGCCACCATGGTGAGCAAGGGCGAG
L7-R	AAAAAGATCTGCTAGCTCGAGTCATGAGGAAGGATGGGTGACCTTCTCCAGCTCATCCTTGTACAGCTCGTCCAT
L8-F	CATCATTTTGGCAAAGAATTCGCCACCATGGTGAGCAAGGGCGAG
L8-R	GAAAAAGATCTGCTAGCTCGAGTCAGGAAGGATGGGTGACCTTCTCCAGCTCATCCTTGTACAGCTCGTCCAT
L9-F	CATCATTTTGGCAAAGAATTCGCCACCATGGTGAGCAAGGGCGAG
L9-R	AAGATCTGCTAGCTCGAGTCAAGGATGGGTGACCTTCTCCAGCTCATCCTTGTACAGCTCGTCCAT

Materials and methods

Vectors, cells, animals, and viruses

SP2/0 (mouse myeloma cell line), DF1 (chicken embryo fibroblast cell line), and HD11 (chicken macrophage cell line) were preserved in the Avian Immunosuppressive Disease Division (referred to as our laboratory), Harbin Veterinary Research Institute (HVRI), Chinese Academy of Agricultural Sciences (CAAS). The prokaryotic expression vector pCold I was purchased from TaKaRa (Shiga, Japan). The eukaryotic expression vector pCAGGS was supplied by Dr. J. Miyazaki of University of Tokyo, Japan. BALB/c mice were purchased from Liaoning Biological Co., Ltd. (Shanghai, China). Animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of HVRI (approval number 230630-01-GR). The infectious bursal disease virus (IBDV) HLJ0504 strain was previously identified in our laboratory (Qi et al., 2011).

Preparation of recombinant chicken NLRP3 protein

The NLRP3 gene from HD11 cells was amplified via RT-PCR using the upstream primer was 5′-ATGGCAGGAGAAGAAAGCACCATCCTCTTAGAAGC-3′; and downstream was 5′-CCGCAGGACACGCAGCTGGCAGCCGGGTTGGCGCA-3′. NLRP3 with a 6-His tag at the C-terminus was subcloned into pCold I, and the recombinant prokaryotic expression plasmid was named pCold-NLRP3. Additionally, the NLRP3 gene with a FLAG tag at the C-terminus was subcloned into pCAGGS, and the eukaryotic expression plasmid was named pCA-NLRP3. To perform prokaryotic expression of chicken NLRP3, the pCold-NLRP3 was transformed into engineered *Escherichia coli* (DE3) and induced via β-d-1-thiogalactoside (0.2 mmol/L) treatment at 20°C for 22 h. The recombinant NLRP3 protein was purified using gel cutting and electroelution and identified via SDS-PAGE and western blotting.

NLRP3 MAb development

The purified NLRP3 protein was emulsified with equal volumes of Freund’s complete adjuvant (Sigma-Aldrich, MO. USA) and then injected subcutaneously at multiple points (100 μg) into 6-week-old female BALB/c mice. Subsequently, immunization was performed every 2 weeks using the same method, and the subsequent immunogen was emulsified with Freund’s incomplete adjuvant (Sigma-Aldrich). One week after the third immunization, serum antibody titers were measured using an indirect enzyme-linked immunosorbent assay (ELISA) plate coated with immunogenic proteins. Three days before the cell fusion experiment, mice with the highest serum antibody titers were selected for enhanced immunity. The mice were euthanized, and the spleen cells were collected and fused with SP2/0 myeloma cells using PEG (P7306; Sigma-Aldrich). The fused cells were cultured in 96-well plates in hypoxanthine-aminopterin-thymidine-selective medium (Sigma-Aldrich). After 7 days, the NLRP3 antibody in the cell supernatant was detected using an indirect ELISA. After two rounds of flow cytometry sorting, positive hybridoma cells secreting NLRP3 antibody were subcloned into single cell lines. Positive hybridoma cells secreting chicken NLRP3 mAb were cultured until the logarithmic growth stage and then stored. In addition, to prepare the mAb ascites, positive hybridoma cells were injected into the abdominal cavity of mice, and

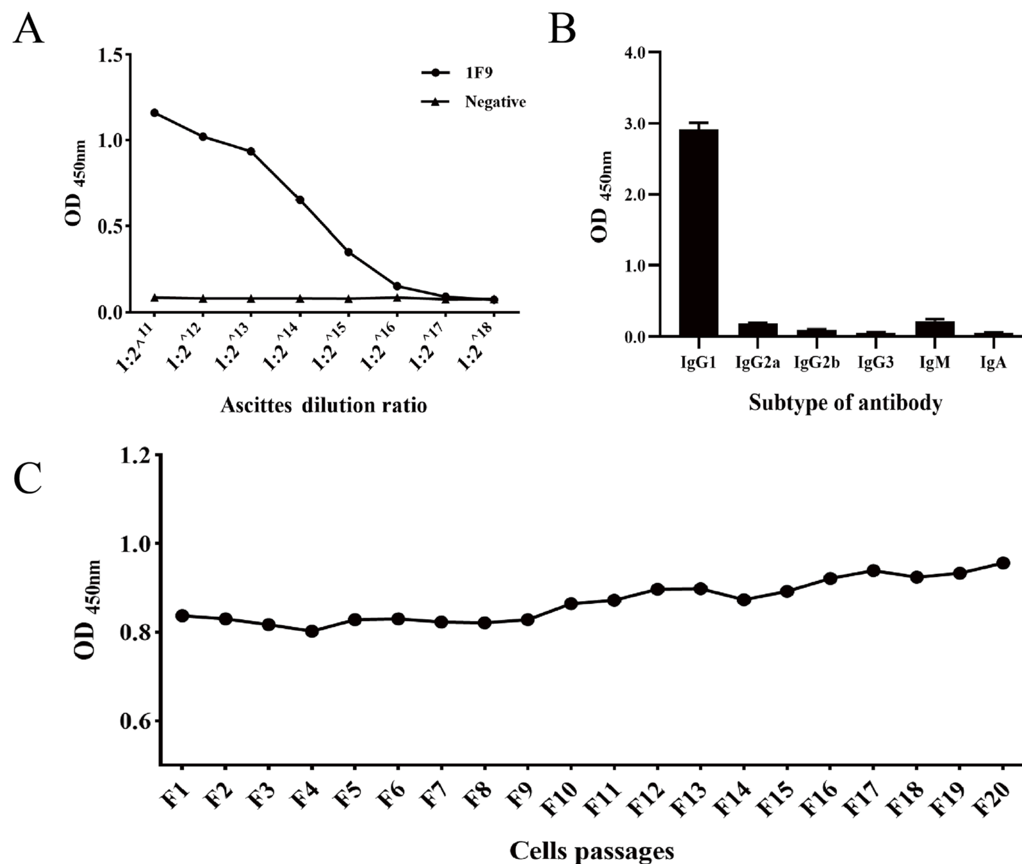

Fig. 1. Production and identification of NLRP3 mAb 1F9. (A) Measurement of ELISA titer of NLRP3 mAb ascites. (B) Identification of the NLRP3 mAb subtype. (C) Detection of NLRP3 mAb secretion by hybridoma cells of different generations.

ascites were collected when the mouse abdomen swelled. The mAb subtype was identified using a mouse mAb subtype ELISA kit (Biodragon, China).

Assessment of stability of MAb secretion by hybridoma cells

The positive hybridomas were passaged continuously for 20 generations. The ELISA titer of the NLRP3 mAb in the cell supernatants of each passage was determined.

Detection of exogenous NLRP3 using the developed MAb

Immunofluorescence assay (IFA) or western blotting was used to assess the recognition of exogenous NLRP3 by the chicken NLRP3 mAb 1F9 developed in this study. For IFA, pCA-NLRP3 was transfected into DF-1 cells for 30 hours, and NLRP3 mAb (1:1000) and fluorescent isothiocyanate-conjugated sheep anti-mouse antibody (Sigma-Aldrich) were used as the primary and secondary antibodies for detection, respectively. For western blotting, pCA-NLRP3 was transfected into HD11 cells for 30 hours. The chicken NLRP3 mAb 1F9, anti-FLAG antibody, or anti-β-actin antibody (Sigma-Aldrich) was used as the primary antibody, and IRDye 800CW goat anti-mouse IgG (LI-COR Biosciences, MA, USA) was used as the secondary antibody for immunoblotting detection. A control group, transfected with pCAGGS, was also established. A similar western blot detection of exogenous chicken NLRP3 was performed in HEK293T cells using the chicken NLRP3 mAb 1F9, a commercial human NLRP3 polyclonal antibody, and a commercial mouse NLRP3 mAb.

Detection of endogenous NLRP3 using MAb

IBDV stimulates macrophages to express endogenous NLRP3. The IBDV HLJ0504 strain was used to stimulate HD11 cells (six-well plate) with 2.5×10^8 copies/well for 12 h. Western blotting was used to detect the recognition of endogenous NLRP3 expression in cells by the chicken NLRP3 mAb 1F9. The FLAG-tag or β-actin antibody was used as the control. In another experiment, HD11 cells were pretreated with lipopolysaccharide (LPS; 1 μg/mL) for 6 h, followed by treatment with 5 mM ATP for 0.5 h, and endogenous NLRP3 was detected using chicken NLRP3 mAb 1F9, human NLRP3 polyclonal antibody, or mouse NLRP3 mAb.

Antigen epitope analysis

To identify the antigenic epitopes recognized by NLRP3 mAb 1F9, the NLRP3 gene was truncated and cloned into the pCAGGS plasmid. An enhanced green fluorescent protein tag was fused to the N-terminus of the truncated NLRP3 gene to construct recombinant eukaryotic expression plasmids. These recombinant plasmids were transfected into DF-1 cells to express truncated NLRP3 proteins. Cells were collected 30 hours post-transfection, and western blotting was performed using NLRP3 mAb 1F9. The construction scheme for NLRP3 truncation is shown in Fig. 3. NLRP3 was truncated into three fragments based on its structural domain: PYD (aa 6-88), NACHT (aa 170-355), and LRR (aa 620-734). The PYD fragment was further divided into four fragments: P1 (aa 6-26), P2 (aa 21-47), P3 (aa 42-68), and P4 (aa 63-88). Next, P3 was truncated into six fragments: L1 (aa 42-66), L2 (aa 42-64), L3 (aa 42-62), R1 (aa 44-68), R2 (aa 46-68), and R3 (aa 48-68). We further truncated L3 into six fragments: L4 (aa 42-59), L5 (aa 42-56), L6 (aa 42-53), L7 (aa 42-52), L8 (aa 42-51), and L9 (aa 42-50). The primers used to construct

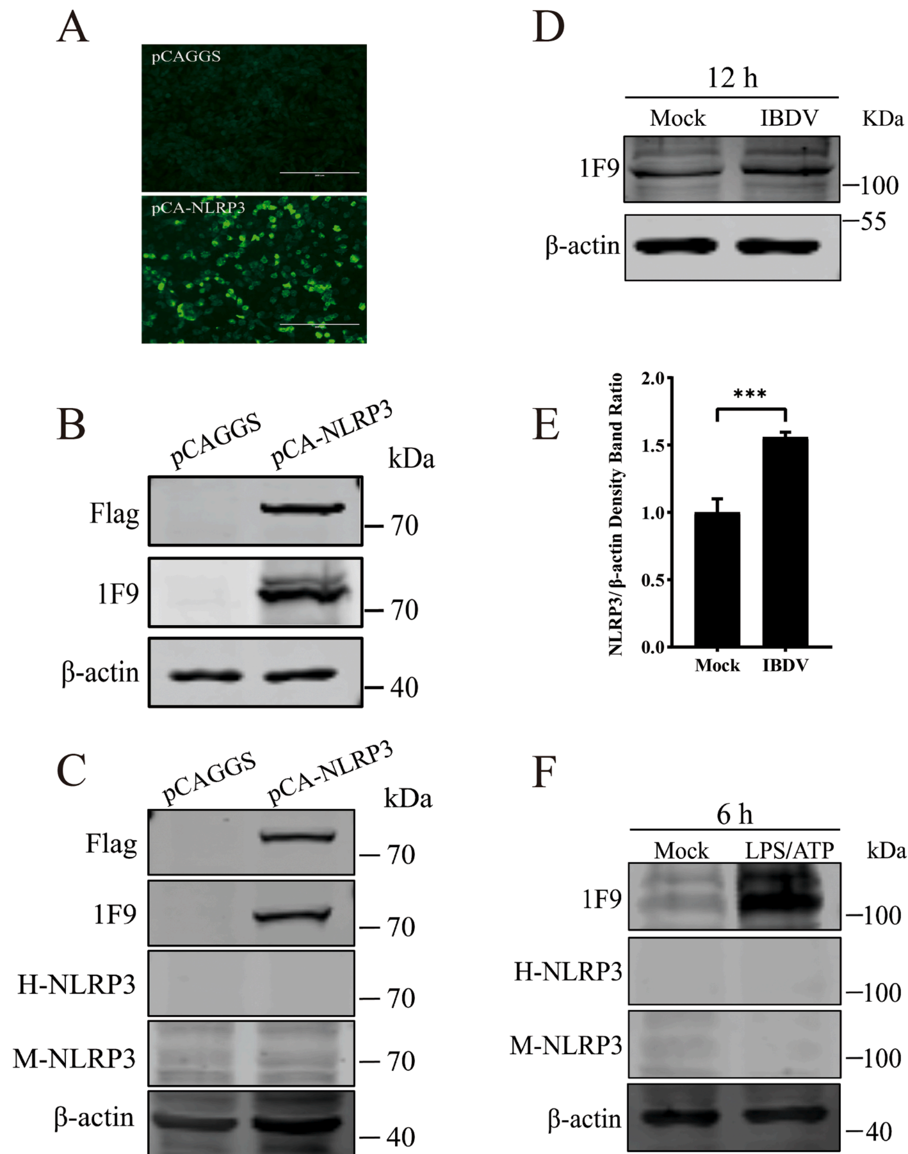

Fig. 2. Detection of different types of NLRP3 by mAb 1F9. (A) IFA detection of exogenous NLRP3 in pCA-NLRP3- or pCAGGS-transfected DF-1 cells using NLRP3 mAb 1F9. (B) Western blot detection of exogenous NLRP3 in pCA-NLRP3- or pCAGGS-transfected HD11 cells using NLRP3 mAb 1F9, anti-FLAG mAb, and anti-β-actin mAb. (C) Western blot detection of exogenous NLRP3 in pCA-NLRP3- or pCAGGS-transfected HEK293T cells using NLRP3 mAb 1F9, human NLRP3 polyclonal antibody (abbreviated as H—NLRP3), mouse NLRP3 mAb (abbreviated as M-NLRP3), anti-FLAG mAb, and anti-β-actin mAb. (D) Western blot detection of endogenous NLRP3 in HD11 cells stimulated by IBDV using NLRP3 mAb 1F9 and anti-β-actin mAb 12 h post-stimulation. (E) Relative grayscale value of NLRP3 protein in D. (F) Western blot detection of endogenous NLRP3 in HD11 cells stimulated by LPS/ATP using NLRP3 mAb 1F9, H—NLRP3 antibody, M-NLRP3 mAb, and anti-β-actin mAb 6 h post-stimulation.

truncated NLRP3 are listed in Table 1.

Sequence alignment and spatial structure analysis of the antigen epitope

The antigen epitope sequences of chicken NLRP3 from different species selected from the National Center for Biotechnology Information database (https://www.ncbi.nlm.nih.gov/) were compared. The GenBank accession numbers used for comparison were as follows: mallards (MH373356.1), domestic cattle (NM_001102219.1), horses (NC_009157.3), pigs (JQ219660), Norwegian rats (NM_001191642.1), chimpanzees (NC_072398.2), and humans (NM_001079821.3). The three-dimensional structure of chicken NLRP3 was predicted using SWISS-MODEL (https://swissmodel.expasy.org/) to determine the spatial location of the antigen epitope.

Results

Development of hybridoma cells secreting chicken NLRP3 MAb

The chicken NLRP3 gene, with a length of 2205 bp (encoding 734 amino acids), was amplified, and the chicken NLRP3 protein (approximately 87 kDa) was prepared using a prokaryotic expression system. Using hybridoma cell technology and flow cytometry sorting, a hybridoma cell line (NLRP3-mAb-1F9, abbreviated as 1F9) capable of secreting chicken NLRP3 mAbs was developed. MAb ascetic fluid was also prepared, and the ELISA titer was 1:2^{15} (Fig. 1A). The subtype of mAb 1F9 was identified as IgG1 (Fig. 1B). Blind-passage data showed that the hybridoma cell line 1F9 could stably secrete chicken NLRP3 mAbs from F1 to F10 (Fig. 1C).

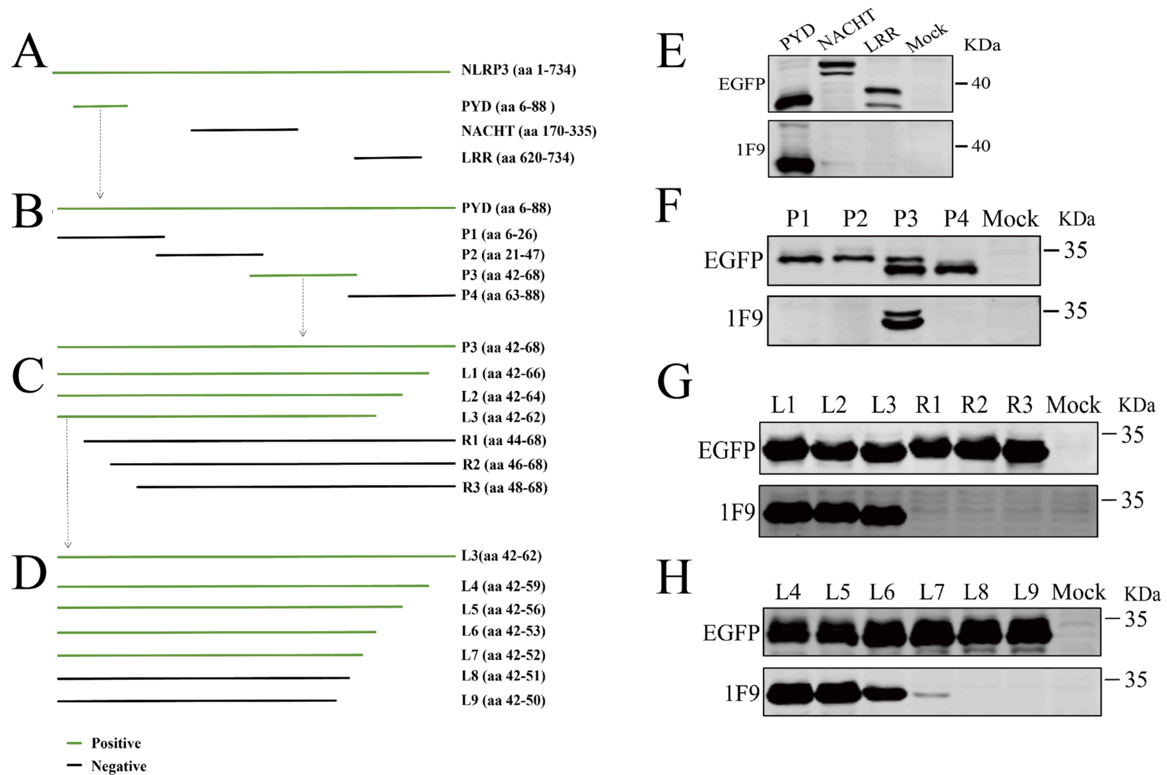

Fig. 3. Antigen epitope identification in chicken NLRP3 recognized by mAb 1F9. (A-D) Schematic diagrams of the truncated overlapping peptides spanning the full length of the NLRP3 protein and the sequential amino acid deletion for fine mapping. Fragments of NLRP3 that can be recognized by mAb 1F9 are highlighted in green. (E-H) Identification of the NLRP3 epitope recognized by mAb 1F9 using western blotting. E-H correspond to A-D, respectively.

Detection of exogenous or endogenous NLRP3 using the MAb

To analyze the practicality of mAb 1F9, the recognition of endogenous or exogenous NLRP3 by mAb 1F9 was detected by IFA and western blotting. IFA results (Fig. 2A) showed specific green fluorescence in DF-1 cells transfected with pCA-NLRP3 and no fluorescence in the empty vector pCAGGS-transfected group. Western blot data (Fig. 2B) showed that in the pCA-NLRP3 transfected-HD11 cells, mAb 1F9 could recognize the endogenous NLRP3 protein (approximately 87 kDa) and that the FLAG-tag antibody could also recognize this band, whereas in the pCAGGS transfection group, the specific band was not detected by mAb 1F9 or the FLAG-tag antibody. In a similar western blot analysis of exogenous chicken NLRP3 performed in HEK293T cells, mAb 1F9 recognized the endogenous NLRP3 protein, whereas the human NLRP3 polyclonal antibody or mouse NLRP3 mAb did not (Fig. 2C).

For endogenous NLRP3 detection, western blotting showed that compared with the mock cell group, NLRP3 protein in HD11 cells upregulated by IBDV infection was recognized by mAb 1F9 (Fig. 2D, E). In another experiment, compared with the mock cell group, NLRP3 protein in HD11 cells upregulated by LPS/ATP was also recognized by mAb 1F9 but could not be detected by the human NLRP3 polyclonal antibody or mouse NLRP3 mAb (Fig. 2F). These results indicate that the prepared NLRP3 mAb can be used to detect the eukaryotic expression of NLRP3 through IFA and western blotting.

Identification of NLRP3 epitope recognized by MAb 1F9

Western blotting was performed on serially truncated NLRP3 peptides (Fig. 3). In the first round of detection, mAb 1F9 specifically recognized PYD (aa 6-88) (Fig. 3A, E). The results of the second round of detection showed that the epitope recognized by mAb 1F9 was located at P3 (aa 42-68) in the PYD domain (Fig. 3B, F). In the third round of detection, P3 was no longer recognizable to mAb 1F9 when its N-terminal amino acid was removed but remained recognizable when its C-terminal amino acid was gradually truncated by six amino acids (Fig. 3C, G). Further truncation of the C-terminal amino acid of P3 revealed that the epitope recognized by mAb 1F9 was 42DELEKVTHPSS52 of chicken NLRP3 (Fig. 3D, H).

Bioinformatics analysis of the antigen epitope

As shown in Fig. 4A, the newly identified antigen epitope 42DELEKVTHPSS52 has low homology with mallard and even lower homology with mammals. Three-dimensional structural analysis revealed that the antigen epitope of 42DELEKVTHPSS52 was located in the PYD domain of chicken NLRP3 (Fig. 4B).

Discussion

Natural immunity exists in all multicellular animals and is one of the oldest known anti-infection mechanisms (Lauzon, et al., 2007; Tarakhovsky, et al., 2012). As a key factors in natural immunity, natural immune receptors can recognize various endogenous and exogenous factors. To date, four types of natural immune receptors have been discovered, the largest being the NOD-like pattern recognition receptor family. NLRP3 is an important member of this family and can recognize DAMPs or PAMPs and subsequently activate a series of downstream signaling pathways, thereby causing an inflammatory response to protect the body (Mangan, et al., 2018; Huang, et al., 2021; Harris, et al., 2022; Yue, et al., 2023). As an intracellular sensor, NLRP3 is activated by various endogenous and exogenous stimuli including LPS, Nigerian bacteriocin, extracellular ATP, and potassium efflux (Wang, et al., 2021; Zeng, et al., 2022). It plays important roles in host resistance to bacterial, fungal, and viral infections. The excessive activation and abnormal regulation of NLRP3 are closely associated with various diseases (Cabral, et al., 2025)

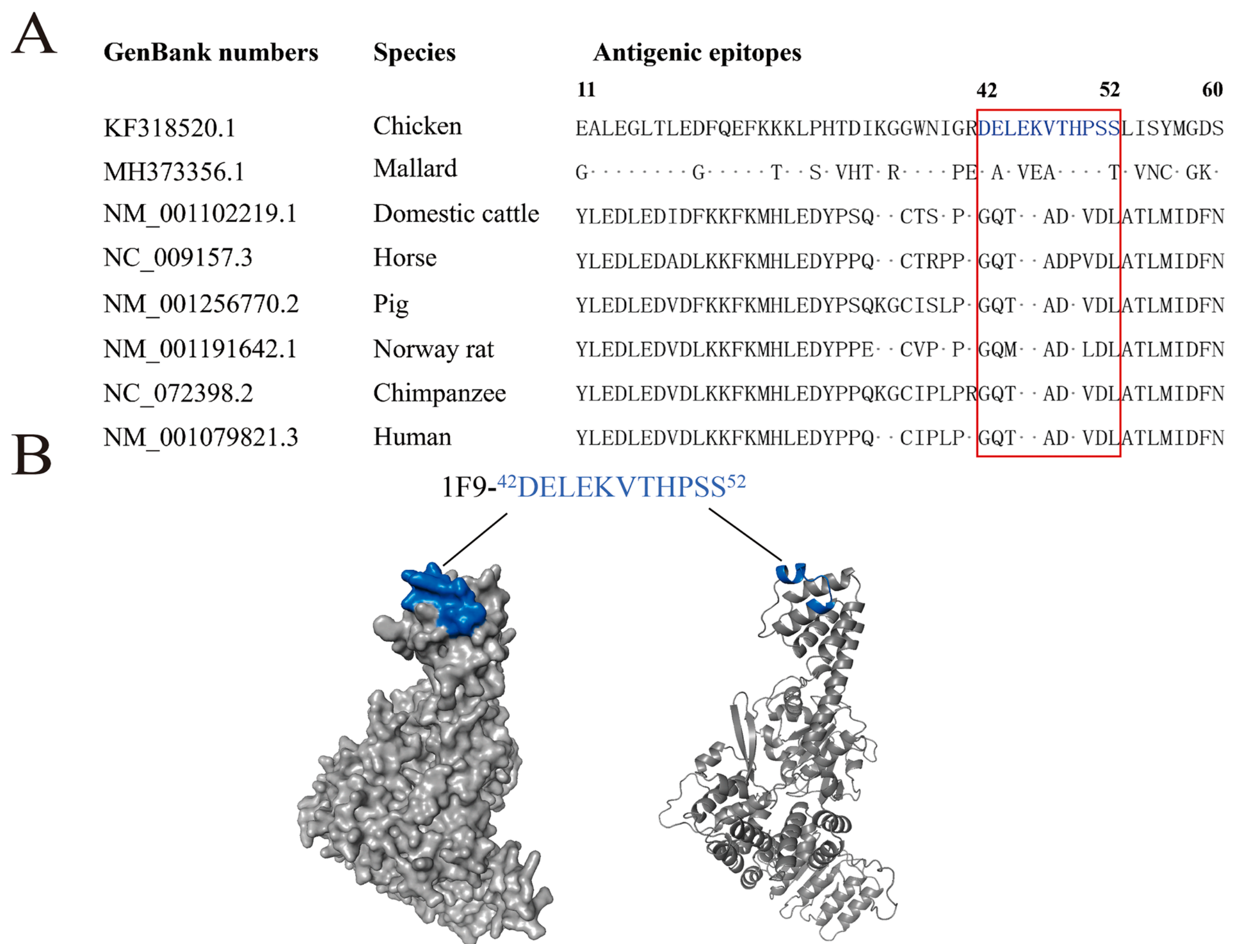

Fig. 4. Analysis of the identified epitope recognized by NLRP3 mAb 1F9. (A) Sequence alignment of the identified epitopes among different species. (B) Localization of the epitope on the predicted 3D model of chicken NLRP3.

Recently, the inflammatory response mediated by the chicken NLRP3 inflammasome has been studied in various pathogenic infections (Gao, et al., 2020; Chen, et al., 2020; He, et al., 2021; Peng, et al., 2022). A previous study showed that extracellular vesicles produced by avian pathogenic *E. coli* activate macrophage pro-inflammatory responses and neutrophil extracellular traps through the NLRP3 inflammasome via the TLR4/MYD88/NF-κB signaling pathway. In addition, avian adenovirus type 4 can induce NLRP3 inflammasome activation in HD11 cells, thereby regulating the secretion of IL-1β (Wang, et al., 2023). Recently, *Lactiplantibacillus plantarum* postbiotics were found to protect broilers from *Salmonella* infection by inhibiting the NLRP3 inflammasome (Guan, et al., 2024). To date, host factor antibodies for natural immunology research available on the market have mostly targeted human- or mouse-derived proteins (Wang, et al., 2022). The scarcity of antibodies against chicken-derived proteins limits the depth of the related research.

In this study, we prepared a highly reactive chicken NLRP3 mAb, 1F9, which can recognize the prokaryotic and eukaryotic expression of chicken NLRP3 and can be used for IFA and western blot detection, meeting the basic requirements for chicken inflammation-related research. This study also preliminarily evaluated the practicality of the prepared NLRP3 mAb for inflammation caused by avian pathogens. Acute inflammation is a typical pathogenic process for chicken IBD caused by IBDV (Huang, et al., 2025; He, et al., 2021). Recently, the enhancement of MyD88 oligomerization by viral VP2 has been reported to play an important role in the inflammatory priming stage of IBDV infection (Huang, et al., 2025). However, the molecular mechanism by which the NLRP3 inflammasome functions during the subsequent inflammatory activation stage remains unclear. In the present study, we used IBDV infection to detect chicken NLRP3 production in HD11 cells. Western blot results showed that this NLRP3 mAb could recognize and detect the upregulation of endogenous NLRP3 expression in HD11 cells 12 hours after IBDV inoculation. The development of antibodies against chicken-derived proteins, including NLRP3, will enable systematic analysis of the inflammatory mechanism of IBDV.

In addition, the prokaryotic and eukaryotic chicken NLRP3 detected by chicken NLRP3 mAb 1F9 could not be recognized by a human NLRP3 polyclonal antibody or a mouse NLRP3 mAb. To investigate the underlying reason, we investigated the antigenic epitope recognized by chicken NLRP3 mAb 1F9 and, for the first time, identified 42DELEKVTHPSS52 in the PYD domain as a new antigenic epitope of chicken NLRP3. Antigen epitopes are specific binding sites on antigen molecules that interact with antibodies and determine antigen specificity. This antigen epitope, 42DELEKVTHPSS52, is unique to chickens and has low homology with mallard genes, demonstrating great value for distinguishing chicken NLRP3 from that of other species.

In summary, this study developed a chicken NLRP3 mAb using hybridoma cell technology and identified a novel antigen epitope, 42DELEKVTHPSS52, in the PYD domain of chicken NLRP3 that is recognized by this mAb. This study is valuable for further clarifying the antigen structure and biochemical characteristics of chicken NLRP3.

CRediT authorship contribution statement

Mengmeng Xu: Data curation, Formal analysis, Resources, Software, Validation, Writing – original draft. **Mengmeng Huang:** Supervision. **Guodong Wang:** Methodology, Software. **Jingzhe Han:**

Investigation, Visualization. **Hangbo Yu:** Resources, Software. **Yulong Zhang:** Conceptualization, Formal analysis, Methodology. **Runhang Liu:** Methodology. **Ziwen Wu:** Investigation. **Hongyu Cui:** Methodology. **Yanping Zhang:** Methodology, Resources. **Suyan Wang:** Funding acquisition, Project administration. **Yongzhen Liu:** Funding acquisition, Methodology, Project administration. **Yuntong Chen:** Funding acquisition, Project administration, Resources. **Yulu Duan:** Supervision. **Liuan Li:** Supervision. **Yulong Gao:** Conceptualization, Funding acquisition, Project administration, Resources. **Xiaole Qi:** Writing – review & editing, Conceptualization, Funding acquisition, Project administration.

Disclosures

The authors declare that they have no conflict of interest. No benefits in any form have been or will be received from any commercial party related directly or indirectly to the subject of this manuscript.

Acknowledgments

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