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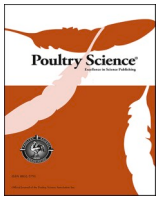

Full-Length Article

Monoclonal antibody development and antigenic epitope identification of chicken pro-IL-1β

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ABSTRACT

Pro-IL-1β is an important inflammatory factor and is also a biomarker for detecting early pro-inflammatory immune responses. However, commercially available antibodies against chicken inflammatory factors are lacking, which prohibits an in-depth exploration of the mechanism of chicken inflammation. This study cloned and expressed chicken pro-IL-1β, and developed a hybridoma cell line 1E12 capable of stably secreting chicken pro-IL-1β monoclonal antibody (mAb). The secreted mAb 1E12 can recognize exogenous or endogenous chicken pro-IL-1β by Western blot and IFA techniques, and for the first time, a novel antigen epitope ^{13}SSLSEETFY^{21} of chicken pro-IL-1β recognized by this mAb was identified. This study not only provides an important tool for the detection and research of chicken pro-IL-1β, but also has significant implications for understanding the antigenic structure and biochemical characteristics of chicken pro-IL-1β.

Introduction

Inflammatory responses are host cell immune responses mediated by inflammasomes triggered by pathogens or other stimuli. The NOD-like receptor thermal protein domain associated protein 3 (NLRP3) inflammasome mediated inflammation is currently the most widely studied type of inflammation (Yilmaz et al., 2015; Pan et al., 2021; Liu et al., 2022; Liu et al., 2023). Infection of chicken flocks with avian diseases can also activate NLRP3 inflammasomes, promote the mature release of interleukin-1β (IL-1β), and trigger inflammatory responses (Black et al., 1989; Yazdi et al., 2016). IL-1β is a pro-inflammatory cytokine mainly produced by monocytes, macrophages, and lymphocytes. It is the central mediator of inflammatory responses and immune regulation in the body, mediating various inflammatory reactions, inducing gene expression and secretion of pro-inflammatory cytokines, and playing a very important role in the pathogenesis of inflammatory diseases (Black et al., 1989). IL-1β precursor (pro-IL-1β) is the pre-cleaved form of mature IL-1β. As early as 1989, it was discovered that pro-IL-1β, a pro-inflammatory cytokine, can be cleaved by the protease Caspase-1 to produce mature IL-1β (Yazdi et al., 2016). The detection of whether pro-IL-1β has been cleaved is often used to determine whether there is an inflammatory response in the body. Therefore, pro-IL-1β plays an important role in initiating early pro-inflammatory immune responses and is also a biomarker for detecting early pro-inflammatory immune responses (Triantafilou., 2021).

At present, the inflammatory mechanism of avian disease pathogens remains poorly understood, and most studies of them focus on fluorescence quantitative detection of inflammatory factors. The lack of chicken-derived inflammatory factor antibodies is an important bottleneck in research of avian diseases. This study cloned and expressed the

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Table 1
Primers.

Primers	Sequence (5′-3′)
P1-F	TTGGCAAAGAATTCGGCGGGGGTAGCATGGCGTTCGTTCCCGACC
P1-R	ATCTGCTAGCTCGAGCTATTTGTCATCGTCATCTTTATAATCGCTACCCCCGCCGGTGACGGGCTCAAAAACCT
P2-F	TTTGGCAAAGAATTCGGCGGGGGTAGCGTTTTTGAGCCCGTCACCTTCCA
P2-R	AAGATCTGCTAGCTCGAGCTATTTGTCATCGTCATCTTTATAATCGCTACCCCCGCCGGTGCCGCTCATCACAC
P3-F	TTTGGCAAAGAATTCGGCGGGGGTAGCGCACTGGGCATCAAGGGCTACAA
P3-R	ATCTGCTAGCTCGAGCTATTTGTCATCGTCATCTTTATAATCGCTACCCCCGCCGCGCCCACTTAGCTTGTAGG
P1-1-F	GCATGGACGAGCTGTACAAGGCGTTCGTTCCCGACCTGGA
P1-1-R	GAAAAAGATCTGCTAGCTCGAGTCAGGGGCCGTAGAAGGTCTCTT
P1-2-F	GCATGGACGAGCTGTACAAGGAGACCTTCTACGGCCCCTC
P1-2-R	GAAAAAGATCTGCTAGCTCGAGTCACACCTGCACGTCCACTGTGGTGTG
P1-3-F	TGGACGAGCTGTACAAGACAGTGGACGTGCAGGTGACGG
P1-3-R	GAAAAAGATCTGCTAGCTCGAGTCAGGTCATGGCCACCACCAGCACGGCG
P1-4-F	CATGGACGAGCTGTACAAGCTGGTGGTGGCCATGACCAAA
P1-4-R	GAAAAAGATCTGCTAGCTCGAGTCACTCCTCCAGGAGCGCGCTCAGGTCGCTG
C1-F	ATTTTGGCAAAGAATTCGCCACCATGGTGAGCAAGGGCGAGG
C1-R	AAAGATCTGCTAGCTCGAGTCAGCCGTAGAAGGTCTCTTCGCTGAGGCTGCTGCTCTCCAGCACGTCCAGGTCGGGAACGAACGCCTTGT
C2-F	ATTTTGGCAAAGAATTCGCCACCATGGTGAGCAAGGGCGA
C2-R	AAAGATCTGCTAGCTCGAGTCAGTAGAAGGTCTCTTCGCTGAGGCTGCTGCTCTCCAGCACGTCCAGGTCGGGAACGAACGCCTTGTACAGCTCGTC
C3-F	TTTGGCAAAGAATTCGCCACCATGGTGAGCAAGGGCGAG
C3-R	AAAGATCTGCTAGCTCGAGTCAGAAGGTCTCTTCGCTGAGGCTGCTGCTCTCCAGCACGTCCAGGTCGGGAACGAACGCCTTGTACAGCT
C4-F	ATTTTGGCAAAGAATTCGCCACCATGGTGAGCAAGGGCGA
C4-R	AAAGATCTGCTAGCTCGAGTCAGGTCTCTTCGCTGAGGCTGCTGCTCTCCAGCACGTCCAGGTCGGGAACGAACGCCTTGTACAGCTCGTC
C5-F	ATTTTGGCAAAGAATTCGCCACCATGGTGAGCAAGGGCGA
C5-R	AAAGATCTGCTAGCTCGAGTCACTCTTCGCTGAGGCTGCTGCTCTCCAGCACGTCCAGGTCGGGAACGAACGCCTTGTACAGCTCGTC
T1-F	TTGGCAAAGAATTCGCCACCATGGTGAGCAAGGGCGAGG
T1-R	AGATCTGCTAGCTCGAGTCAGTAGAAGGTCTCTTCGCTGAGGCTGCTGCTCTCCAGCACGTCCAGGTCGGGAACCTTGTACAGCTCGTC
T2-F	TTGGCAAAGAATTCGCCACCATGGTGAGCAAGGGCGA
T2-R	AGATCTGCTAGCTCGAGTCAGTAGAAGGTCTCTTCGCTGACTGCTGCTCTCCAGCACGTCCAGGTCCTTGTACAGCTCGT
T3-F	TTGGCAAAGAATTCGCCACCATGGTGAGCAAGGGCGA
T3-R	GATCTGCTAGCTCGAGTCAGTAGAAGGTCTCTTCGCTGAGGCTGCTGCTCTCCAGCACGTCCTTGTACAGCTCGTC
T4-F	TTGGCAAAGAATTCGCCACCATGGTGAGCAAGGGCGA
T4-R	AGATCTGCTAGCTCGAGTCAGTAGAAGGTCTCTTCGCTGAGGCTGCTGCTCTCCAGCTTGTACAGCTCGTC
T5-F	TTGGCAAAGAATTCGCCACCATGGTGAGCAAGGGCGA
T5-R	AGATCTGCTAGCTCGAGTCAGTAGAAGGTCTCTTCGCTGAGGCTGCTGCTCTCCTTGTACAGCTCGTC
T6-F	TTGGCAAAGAATTCGCCACCATGGTGAGCAAGGGCGA
T6-R	AGATCTGCTAGCTCGAGTCAGTAGAAGGTCTCTTCGCTGAGGCTGCTGCTCTTGTACAGCTCGTC
T7-F	TTGGCAAAGAATTCGCCACCATGGTGAGCAAGGGCGA
T7-R	GAAAAAGATCTGCTAGCTCGAGTCAGTAGAAGGTCTCTTCGCTGAGGCTGCTGTGAGCAAGGGCGAGG
T8-F	TTGGCAAAGAATTCGCCACCATGGTGAGCAAGGGCGAGG
T8-R	GAAAAAGATCTGCTAGCTCGAGTCAGTAGAAGGTCTCTTCGCTGAGGCTCTTGTACAGCTCGTC

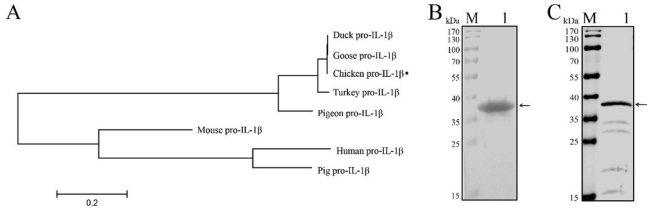

Fig. 1. Identification of the chicken pro-IL-1β gene and its recombinant protein. (A) Phylogenetic tree of pro-IL-1β gene nucleotide sequences from chicken and other species. The chicken pro-IL-1β gene was highlighted by a solid dot. (B) SDS-PAGE identification of the purified prokaryotic expression protein pro-IL-1β. (C) Western blot identification of the pro-IL-1β protein using anti-His monoclonal antibody (mAb).

chicken pro-IL-1β, and prepared a hybridoma cell line capable of stably secreting chicken pro-IL-1β monoclonal antibody (mAb). A series of identification experiments of chicken pro-IL-1β mAb were undertaken, and for the first time, an antigenic epitope of chicken pro-IL-1β was identified. This study is significant for further exploration of the molecular mechanism of inflammation triggered by avian pathogens.

Materials and methods

Vectors, cells, animals, and viruses

The prokaryotic expression vector pCold I was purchased from TaKaRa. The eukaryotic expression vector pCAGGS was kindly supplied by Dr. J. Miyazaki, University of Tokyo, Japan. Chicken embryo fibroblast cell line DF-1, chicken macrophage cell line HD11, and mouse myeloma cell line SP2/0 were preserved in the Avian Immunosuppressive Disease Division (referred to as our laboratory), Harbin Veterinary Research Institute, the Chinese Academy of Agricultural Sciences. BALB/c mice were purchased from Liaoning Biological Co., LTD. The animal experiment has been approved by the Institutional Animal Care and Use Committee (IACUC) of Harbin Veterinary Research Institute with the approval number of 230630-01-GR. Infectious bursal disease virus (IBDV) HLJ0504 strain (Qi et al., 2011) were isolated and identified by our laboratory.

Preparation of the recombinant pro-IL-1β protein of chicken

With RT-PCR, the chicken pro-IL-1β gene was amplified from HD11 cell and sub-cloned into pCold I using a homologous recombination assay kit (C115-1, Novozan, China). The C-terminal of pro-IL-1β was fused with a 6 His tag. The upstream primer was 5′-ATGGCGTTCGTTCCCGACCTGGACG-3′; the downstream primer was 5′-TCAGCGCCCACTTAGCTTGTAGGTG-3′. The correct recombinant prokaryotic expression plasmid identified by sequencing was named pCold-pro-IL-1β. In addition, the recombinant eukaryotic expression plasmid pCAGGS-pro-IL-1β was constructed, of which the chicken pro-IL-1β gene fused with a Flag tag at its C-terminal was sub-cloned into pCAGGS.

Homology comparison analysis was conducted between the pro-IL-1β gene of chicken and the pro-IL-1β genes of human (GenBank accession number: M15840), duck (DQ393268), goose (DQ393269), turkey

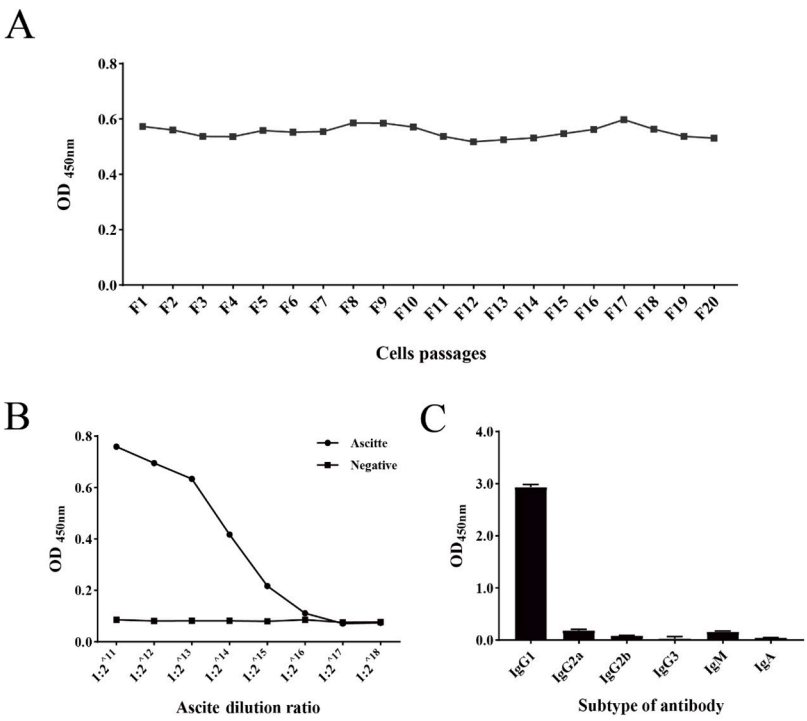

Fig. 2. Production and identification of the pro-IL-1β mAb 1E12. (A) Detection of the pro-IL-1β mAb secretion by hybridoma cells of different generations. (B) Detection of ELISA titer of the pro-IL-1β mAb ascites. (C). Identification of the pro-IL-1β mAb subtype.

(DQ393271), pigeon (DQ393270), mouse (M15131), and pig (NM214055), and a gene nucleotide sequence evolutionary tree was constructed using MAGA6.0 software.

The pCold-pro-IL-1β was transformed into the bacterium *E.coli* (DE3) (TaKaRa). After inducing by β-D-1-thiogalactoside (IPTG) (0.2 mmol/L) at 20°C for 22 h, the recombinant protein was harvested and then purified by gel-cutting and electroelution. The harvested recombinant pro-IL-1β protein was identified by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot.

Development of hybridoma cells secreting chicken pro-IL-1β MAb

The mAb against pro-IL-1β was prepared using hybridoma technology. The purified pro-IL-1β protein was emulsified in equal volume with Freund's complete adjuvant or Freund's incomplete adjuvant (Sigma-Aldrich (Shanghai) Trading Co. Ltd., Shanghai, China) and then injected into 6-week-old female BALB/c mice by multi-point subcutaneous injection (100 μg). Subsequently, two more immunizations were administered, with a 2-week interval between each vaccination. On day-7 post immunization, the antibody titer in serum was detected by indirect ELISA coated with the prokaryotic expression pro-IL-1β protein. Three days before cell fusion experiments, mice with the highest antibody titer of serum were selected for booster immunization. Mice were then euthanized and spleen cells were collected and fused with SP2/0 myeloma cells at 7:1 by PEG (P7306, sigma) (Sigma-Aldrich (Shanghai) Trading Co. Ltd., Shanghai, China). The fusion cells were cultured in a 96-well plate with HAT selective medium. Seven days after cell fusion, the anti-pro-IL-1β antibody in the cell supernatant was detected by indirect ELISA. The positive hybridoma cells secreting pro-IL-1β mAb were sub-cloned into single cell line by two rounds of cell flow cytometry sorting. To further prepare mAb ascites, 6-week-old female BALB/c mice were intraperitoneally injected with hybridoma cells after 7 d of sensitization with Freund's incomplete adjuvant. Approximately 7 d later the mAb ascites were collected and preserved at -80 °C.

Identification of the stability of antibody secretion ability in hybridoma cells

The positive hybridoma cells were passaged continuously for 20 generations, and the mAb titer in the cell supernatants of each passage was detected by indirect ELISA, as described above.

Identification of pro-IL-1β MAb subtype

The subtype of the pro-IL-1β mAb were identified using ELISA kit for identifying Ig subtype of mouse monoclonal antibodies (Biodragon Biotechnology Co., Ltd., Suzhou, China).

Detection of recognition of exogenous pro-IL-1β by the MAb

The detection of recognition of exogenous pro-IL-1β by the pro-IL-1β mAb was performed using Western blot and IFA. The pCAGGS-pro-IL-1β was transfected into HD11 cells for 30 h. Pro-IL-1β mAb (1:1000) was used as the primary antibody, and IRDye 800CW Goat anti-Mouse IgG (Licor (Harbin) Trading Co. Ltd., Harbin, China) was used as the secondary antibody for Western blot detection. In addition, the recombinant eukaryotic expression plasmid pCAGGS-pro-IL-1β was transfected into DF-1 cells for eukaryotic expression of pro-IL-1β. After transfection for 30 h, IFA detection was performed using pro-IL-1β mAb (1:1000) as the primary antibody and FITC-labelled goat anti-mouse IgG (Sigma−Aldrich (Shanghai) Trading Co. Ltd., Shanghai, China) as the secondary antibody.

Detection of recognition of endogenous pro-IL-1β by the MAb

Lipopolysaccharide (Yu et al., 2017) and infectious bursal disease virus (Liu et al., 2019) can stimulate macrophages to express endogenous pro-IL-1β. HD11 cells were stimulated with LPS at a dose of 1 μg/mL for 6 h, and the pro-IL-1β mAb (1:1000) was used as the primary antibody. The recognition of pro-IL-1β mAb to endogenous expression of pro-IL-1β in cells was detected by IFA and Western blot, respectively. In

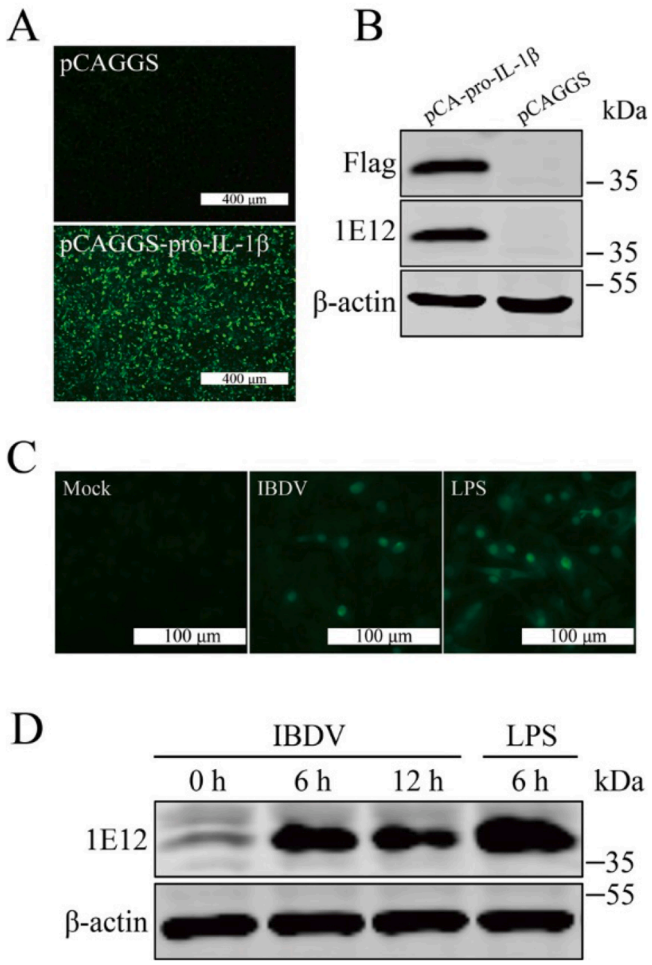

Fig. 3. Detection of different types of pro-IL-1β by mAb. (A) IFA detection of exogenous pro-IL-1β in DF-1 cells transfected with pCA-pro-IL-1β or pCAGGS plasmid with pro-IL-1β mAb. (B) Western blot detection of exogenous pro-IL-1β in HD11 cells transfected with pCA-pro-IL-1β or pCAGGS plasmid by pro-IL-1β mAb, anti-Flag mAb, and anti-β-actin mAb. (C) IFA detection of endogenous pro-IL-1β in HD11 cells stimulated by IBDV or LPS with the pro-IL-1β mAb 6 h post-stimulation. (D) Western blot analysis of endogenous pro-IL-1β in HD11 cells stimulated by IBDV or LPS with the pro-IL-1β mAb and anti-β-actin mAb at 0, 6, and 12 h post-stimulation.

addition, HD11 cells were stimulated with IBDV at a dose of 2.5×10^8 copies / well (6-well plate) for 6 h or 12 h, IFA or Western blot as above was used to detect the endogenous expression of pro-IL-1β.

Identification of the antigen epitope recognized by the MAb

Chicken pro-IL-1β gene, were truncated and expressed and Western blot was used to identify the antigenic epitopes of pro-IL-1β recognized by the pro-IL-1β mAb 1E12. The flag or EGFP tag were fused at the C-terminus or N- terminus of the truncated pro-IL-1β gene and these were then sub-cloned separately into the pCAGGS plasmid. The recombinant eukaryotic expression plasmid was transfected into DF-1 cells, and after 30 h the pro-IL-1β mAb 1E12, Flag mAb ((Sigma−Aldrich (Shanghai) Trading Co. Ltd., Shanghai, China) or EGFP tag mAb (Santa Cruz (Beijing) Trading Co. Ltd., Beijing, China) were then used to identify the truncated peptide that can be recognized by the mAb 1E12 using Western blot. The primers used for constructing truncated pro-IL-1β are shown in Table 1.

Sequence alignment and spatial structure analysis of the antigen epitope

The antigen epitope sequences of pro-IL-1β among different species selected from NCBI (https://www.ncbi.nlm.nih.gov/) were compared. The 3D structures of chicken pro-IL-1β protein were predicted using online protein structure prediction tool I-TASSER (https://seq2fun.dcmb.med.umich.edu//I-TASSER/), and the identified antigen epitope was located on the structure of pro-IL-1β using PyMol software.

Results

Preparation of chicken pro-IL-1β protein

The chicken pro-IL-1β gene amplified in this study is 804 bp and encodes 267 amino acids. The phylogenetic tree based on the nucleotide sequences of pro-IL-1β genes from different species (Fig. 1A) shows that chicken pro-IL-1β is closely related to ducks, geese, turkeys, and pigeons, with nucleotide homology rates of 99.6 %, 99.6 %, 94.4 %, and 82.2 %, and amino acid homology rates of 100 %, 100 %, 94.7 %, and 78.8 %, respectively. The genetic relationship with humans, mice, and pigs is relatively distant, with nucleotide sequence homology ranging from 38.4 % to 50.7 % and amino acid sequence homology ranging from 9.1 % to 12.1 %.

The chicken pro-IL-1β protein (about 37 kDa) was obtained using a prokaryotic expression system (Fig. 1B), and the recombinant protein was recognized by anti-His mAb (Fig. 1C).

Development of the hybridoma cells secreting chicken pro-IL-1β MAb

A hybridoma cell line capable of secreting chicken pro-IL-1β mAb was obtained using hybridoma cell technology and flow cytometry sorting technology, named pro-IL-1β-mAb-1E12 strain (abbreviated as 1E12 in this article). With blind-passage, the hybridoma cell line 1E12 could stably secrete mAbs from P1 to P20 (Fig. 2A). The mAb ascites were also prepared, of which the ELISA titer of mAb was $1{:}2^{15}$ (Fig. 2B). The mAb 1E12 was identified as subtype IgG1 (Fig. 2C) using a mouse monoclonal antibody isotype ELISA kit.

Detection of recognition of exogenous or endogenous pro-IL-1β by the MAb

To determine the specificity and practicality of the mAb 1E12, the recognition of endogenous or exogenous pro-IL-1β by the mAb 1E12 was detected using Western blot and IFA. The IFA results (Fig. 3A) showed that specific green fluorescence was observed in DF-1 cells transfected with pCAGGS-pro-IL-1β, while no fluorescence was observed in the pCAGGS transfected group. Western blots demonstrated (Fig. 3B), that in the pCAGGS-pro-IL-1β transfection group, mAb 1E12 could recognize a band of approximately 37 kDa, and Flag-tag antibody could also recognize this band. In the empty vector pCAGGS transfection group, specific bands were not detected by mAb 1E12 or Flag-tag antibody, but both sets of samples could detect cell β-actin. These results indicated that the prepared pro-IL-1β mAb can be used for detecting eukaryotic expression protein of pro-IL-1β through IFA and Western blot.

The recognition of endogenous pro-IL-1β by the mAb 1E12 was also evaluated. The IFA results showed that specific green fluorescence was observed in HD11 cells 6 h after IBDV inoculation compared to the mock cell control; green fluorescence was also detected in the LPS stimulation group (Fig. 3C). Western blots showed that compared with the mock cell group, pro-IL-1β was detected in LPS stimulation group for 6 h and IBDV inoculation group for 6 and 12 h (Fig. 3D). This indicates that the prepared pro-IL-1β mAb exhibits good immunoreactivity with endogenous pro-IL-1β protein in HD11 cells.

Precise localization of pro-IL-1β epitope recognized by MAb 1E12

The antigen epitope recognized by the mAb 1E12 was determined

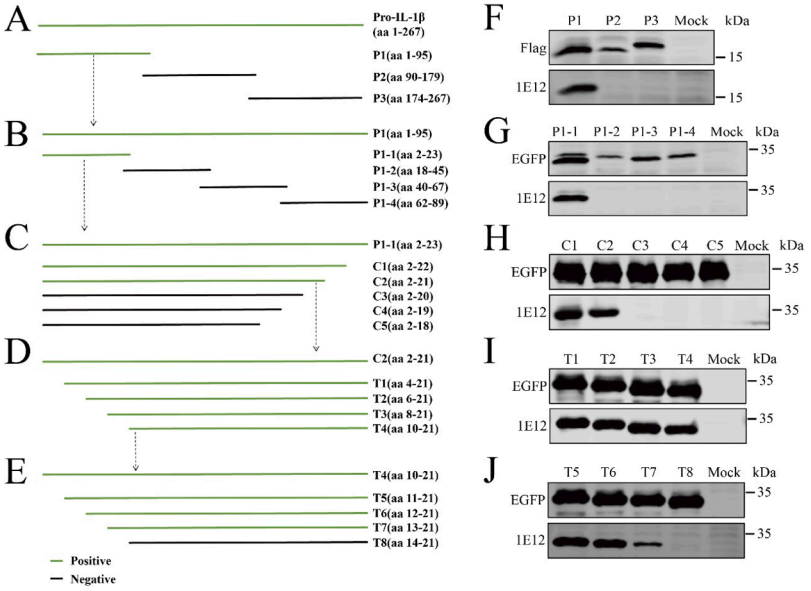

Fig. 4. Antigen epitope identification of chicken pro-IL-1β by mAb 1E12. (A-E) Schematic diagrams of the truncated overlapping peptides spanning the full length of pro-IL-1β protein and the sequential deletion of amino acid for fine mapping. Fragments of pro-IL-1β that can be recognized by mAb 1E12 are highlighted in green. (F-J) Identification of the pro-IL-1β epitope recognized by mAb 1E12 using Western blot. F-J corresponds to A-E, respectively.

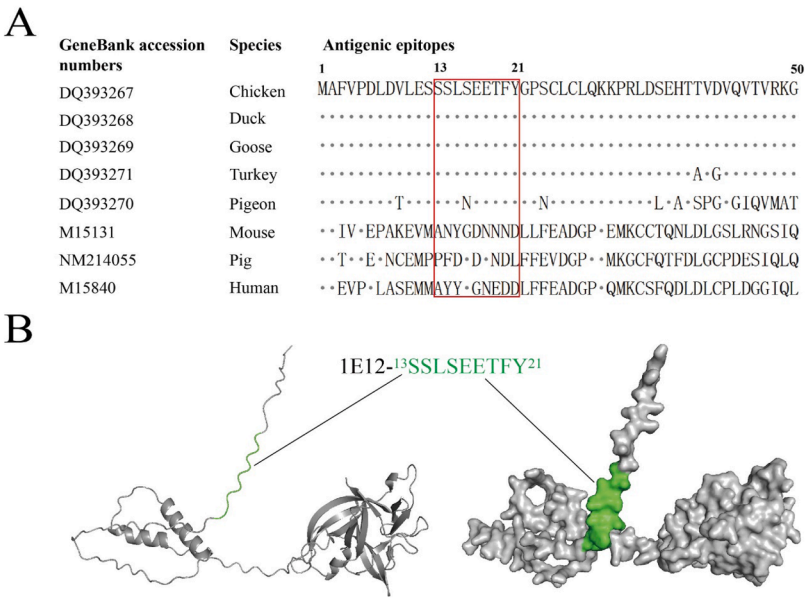

Fig. 5. Analysis of the identified epitope recognized by pro-IL-1β mAb 1E12. (A) Sequences alignment of the identified epitopes among different species. (B) Localization of the epitope on the predicted 3D model of chicken pro-IL-1β.

with series of truncated peptides spanning the full-length of chicken pro-IL-1β, as demonstrated by Western blot analysis (Fig. 4). The pro-IL-1β truncations of P1 (aa 1-95), P2 (aa 90-179), and P3 (aa 174-267) (Fig. 4A) were analyzed first and the results showed that the mAb 1E12 could recognize P1 (aa 1-95) (Fig. 4F). P1-1 (aa 2-23), P1-2 (aa 18-45), P1-3 (aa 40-67), and P1-4 (aa 62-89) (Fig. 4B) were then analyzed and the mAb 1E12 could recognize P1-1 (aa 2-23) (Fig. 4G). To precisely identify the epitope recognized by mAb 1E12, P1-1 was truncated at its C-terminus (Fig. 4C) and C2 (aa 2-21) could be recognized by mAb 1E12 but C3 (aa 2-20) could not (Fig. 4H). C2 (aa 2-21) was further truncated at its N-terminus (Fig. 4D, E) and mAb 1E12 could specifically recognize T7 (aa 13-21) but could not recognize T8 (aa 14-21) (Fig. 4I, J). These results indicated that the ^{13}SSLSEETFY^{21} of pro-IL-1β is the accurate epitope recognized by mAb 1E12.

Bioinformatics analysis of the antigen epitope

The newly identified antigen epitope ^{13}SSLSEETFY^{21} of pro-IL-1β is conserved in poultry including chicken, duck, geese, and turkeys (Fig. 5A). This epitope of chicken differs from that of pigeon by only one amino acid. However, this epitope is almost completely different from mammals (mice, pigs and humans). The spatial structure of pro-IL-1β showed that the 1E12 mAb epitope was located in the leading peptide portion of chicken pro-IL-1β protein (Fig. 5B).

Discussion

At present, commercially available pro-IL-1β antibodies are mostly anti-mouse or anti-human, with low cross reactivity with chicken pro-IL-

1β, and they cannot be directly used for exploring the molecular mechanisms of chicken inflammation (Yu et al., 2020). This is an important reason why related research is relatively lagging behind. This study developed a hybridoma cell line 1E12 that can stably secrete chicken pro-IL-1β mAb. The secreted mAb specifically recognized chicken pro-IL-1β protein and can be used for Western blot and IFA, providing a good tool for chicken-inflammation related research.

To further investigate the practicality of this mAb 1E12 in research and detection, this study validated the effective recognition of endogenous pro-IL-1β in chicken macrophage line HD11 by this mAb following LPS stimulation. Pro-IL-1β is an important pro-inflammatory factor and is often used as a marker for detecting inflammation. The RT-qPCR is often used for preliminary detection of inflammatory factors, but it only reflects gene transcription levels (Carballeda et al., 2015; Aliyu et al., 2022; Ding et al., 2023). Western blot and IFA can detect the abundance of host proteins, reflecting the final state of protein expression (Zhang et al., 2022). These detection methods require host-protein specific antibodies, but antibodies against these chicken-derived proteins are very scarce. The preparation of chicken pro-IL-1β antibody suitable for Western blot and IFA detection is of significance for the study of molecular mechanisms related to inflammation in chickens.

To further evaluate the detection by this pro-IL-1β mAb 1E12 in avian pathogen triggered inflammation, we conducted detection tests in a model of IBDV inoculation with HD11 cells (Ingrao et al., 2013; Dey et al., 2019). Results of Western blot directed by this pro-IL-1β mAb 1E12 showed that IBDV could significantly upregulate the expression of pro-IL-1β in HD11 cells at 6 h and 12 h after inoculation. In the IFA directed by this pro-IL-1β mAb, upregulation of pro-IL-1β expression was also detected in HD11 cells 6 h after IBDV inoculation, consistent with the recognition that IBDV can trigger acute inflammation (Liu et al., 2019). These experimental data validated the practicality of the chicken pro-IL-1β mAb developed in this study. Furthermore, this study identified a novel antigenic epitope ^{13}SSLSEETFY^{21} of chicken pro-IL-1β recognized by mAb 1E12, located in the leading peptide region of chicken pro-IL-1β protein. This epitope is conserved in several common poultry species, which indicates that mAb 1E12 can not only be used for the study of chicken inflammation, but also for the detection of pro-IL-1β in ducks, geese, and turkeys. To our knowledge, this is the first report identifying an antigen epitope of chicken pro-IL-1β.

In summary, this study developed a hybridoma cell line 1E12 that can stably secrete chicken pro-IL-1β mAb. The secreted mAb can recognize exogenous or endogenous chicken pro-IL-1β by Western blot and IFA, and for the first time, a novel antigen epitope ^{13}SSLSEETFY^{21} of chicken pro-IL-1β recognized by this mAb was identified. This study not only provides an important tool for the detection and research of chicken pro-IL-1β, but also has significant implications for understanding the antigenic structure and biochemical characteristics of chicken pro-IL-1β.

Declaration of competing interest

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.

Acknowledgements

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