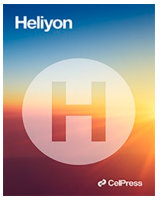
Heliyon 10 (2024) e33145

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Optimization of preparation method and specificity verification of cat CD19 monoclonal antibody for disease diagnosis and treatment

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ARTICLE INFO

Keywords:
B cell malignancies
Cat
CD19 monoclonal antibody

ABSTRACT

CD19 is a surface antigen on B cells that regulates B cell activation and proliferation, participating in B cell signaling. It is expressed in all B cell lineage tumor diseases, making CD19 a significant marker for detecting B cell tumor diseases and an important target for related immunotherapies. In recent years, with the deepening research on canine and feline diseases and the establishment of animal models, the demand for cat CD19 monoclonal antibodies (mAbs) has been steadily increasing. We successfully prepared cat CD19-specific monoclonal antibodies using a KLH-conjugated cat CD19 peptide as an antigen and optimized the antibody production method. The obtained monoclonal antibodies' molecular and cellular affinities were identified using CD19 peptides, eukaryotic overexpressed proteins, and peripheral blood mononuclear cells (PBMCs). The results indicate that the CD19-3H9 and CD19-8A7 monoclonal antibodies prepared in this study specifically bind to the CD19 molecule, demonstrating their suitability for use in ELISA, Western blot, and cell assays. This study successfully produced cat CD19 monoclonal antibodies with specificity and optimized the antibody preparation method, laying the foundation for the diagnosis and targeted drug combination therapy of B cell tumor diseases in both humans and pets.

1. Introduction

CD19 is a transmembrane glycoprotein belonging to the immunoglobulin (Ig) family [1,2], widely distributed on the surface of B cells. It is initially expressed on pro-B cells and disappears after B cell maturation, reappearing when B cells differentiate into plasma cells [3,4]. Numerous in vitro studies suggest that CD19 plays a crucial role in regulating B cell growth and development [5–8]. Functioning as a receptor molecule, CD19 modulates the signal transduction of B cells [9,10]. As a surface antigen specifically recognized by B cells, CD19 is expressed in virtually all malignant B cell tumors. Consequently, CD19-related antibodies play a

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https://doi.org/10.1016/j.heliyon.2024.e33145
Received 13 December 2023; Received in revised form 14 June 2024; Accepted 14 June 2024
Available online 15 June 2024

dominant role in the detection and treatment of B cell tumor diseases [11–13].

Antibodies are widely utilized in biomedical research, including applications such as Western blot, flow cytometry, and immunohistochemical analysis, and have further expanded into the fields of disease diagnosis and immunotherapy [14,15]. Monoclonal antibodies (mAbs) possess advantages of high specificity and sensitivity, making them exceptionally valuable in biological detection and medical research [16]. Monoclonal antibodies are not only extensively applied in human medical research but are increasingly utilized in animal breeding and disease control, such as detecting veterinary drug residues in animal-derived food and diagnosing various diseases in different animal species [17]. In recent years, with the rapid development of biotechnology, antibody production technologies have been continually updated and improved. New antibodies, such as chimeric antibodies and humanized antibodies, have been obtained [18]. Among them, chimeric antigen receptor T cells targeting CD19 (CD19-CAR-T) have achieved significant success in treating B cell tumor diseases [19–21]. Simultaneously, in preclinical and phase I, II clinical trials, excellent therapeutic effects have been demonstrated in B cell acute lymphoblastic leukemia (B-ALL) [11].

With the development and progress of society, there is a growing concern for the health of companion animals, and researchers are delving deeper into the exploration of canine and feline tumor diseases [22,23]. However, there is limited research literature both domestically and internationally on antibodies for canine and feline tumors, especially in the development and preparation of antibodies for detecting and treating feline B cell tumor diseases. Therefore, building upon reported antibody preparation methods, this study developed and validated a murine anti-cat CD19 monoclonal antibody. Further optimization and innovation were performed, laying the research foundation for the diagnosis and treatment of feline B cell tumors and related immune diseases.

2. Materials and methods

2.1. Materials

2.1.1. Cells and experimental animals

SP2/0 cells (CRL-1581) and 293T cells (CRL-3216) were obtained from American Type Culture Collection (ATCC, https://www.atcc.org). SPF BALB/c female mice aged 4–6 weeks were obtained from Beijing VetoLife company and housed in designated experimental facilities in compliance with regulations (CPCSEA approval number: IRM-DWLL-2022242).

2.2. Design of CD19 peptides

Based on the amino acid sequence of Felis catus CD19 and the optimal peptide sequence CGLGNRSSEGPKPSSGYP, the entire protein sequence of feline CD19 was predicted using three epitope prediction software tools: IEDB (http://tools.iedb.org/bcell/), SVMTriP (http://sysbio.unl.edu/SVMTriP/index.php), and bepipred-1.0 (http://www.cbs.dtu.dk/services/bepipred-1.0/). After comprehensively evaluating the predictions from these three software tools, the top-ranked optimal peptide sequence was selected and sent to Beijing Zhongkeyaguang company for the coupling to Keyhole Limpet Hemocyanin (KLH) protein and sequence synthesis. The conjugated peptide with a purity of $\geq$95 % was then used in the experiments.

2.3. Construction of CD19 overexpression vector

Retrieve the cat CD19 cDNA gene sequence from the GenBank website and submit it to Bomade Biotechnology Co., Ltd. for cDNA synthesis. Utilizing primers (Table 1) and 2 × TransTaq® High-Fidelity (HiFi) PCR SuperMix II (-dye) (Transgen, AS131-21), amplify the cat CD19 gene fragment through polymerase chain reaction (PCR). After digestion with QuickCut™ *Xho*I enzyme, the ClonExpress II One Step Cloning Kit (Vazyme Biotech, C112-01) was used to ligate with the pLVX-IRES-eGFP vector, constructing the recombinant vector pLVX-IRES-eGFP-hs-cd19 (hereinafter referred to as cCD19-OE vector), as shown in Fig. 1. Plasmid preparation was performed using a endotoxin-free plasmid extraction kit (TIANGEN, DP117), and the full sequence of the vector was identified and confirmed by sequencing for subsequent cellular experiments.

2.4. CD19 overexpression cell lines were obtained and identified

2.4.1. Lentivirus packaging

The cCD19-OE plasmid, PSPAX2 plasmid, and pMD2.G plasmid were pre-mixed in a mass ratio of 4:3:1. Subsequently, the Transporter 5 Transfection reagent (PEI) (Polyxcience, 26008) was utilized for transfection following the provided instructions. The pre-mixed plasmid was transfected into 293T cells for lentivirus packaging. After 72 h of transfection, the cell supernatant was collected, filtered through a 0.22 μm filter membrane, and the virus titer was determined to be 1.25 × 10^6–7-6.25 × 10^;6–7 TU/ml. The collected virus was then stored at −80 °C for subsequent experiments.

Table 1
Primer sequence.

Primer name	Sequence(5′-3′)
cCD19-HIS-F	taccggactcagatctcgagatgccacctcctcttctc
cCD19-HIS-R	tcagtggtggtggtggtggtgcctagtgctc

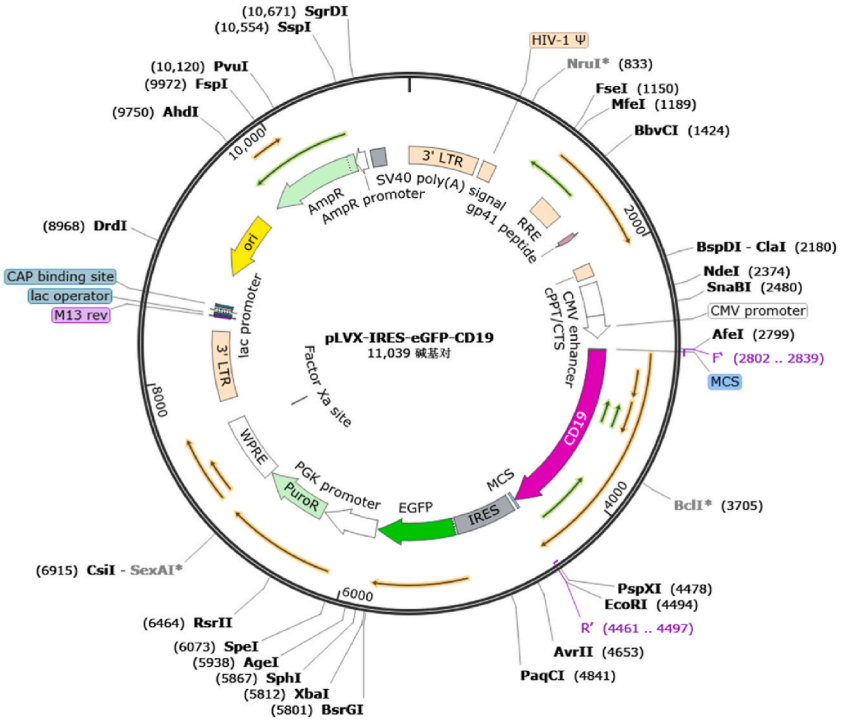

Fig. 1. The chart of recombinant vector pLVX-IRES-eGFP–HIS–CD19.

2.4.2. CD19 overexpression cell population was obtained

293T cells were cultured in 6-well plates until reaching a confluency of 70%–80 % for transfection. Subsequently, 2 ml of the packaged virus solution was added to each well for infection. After 24 h, drug screening was initiated in DMEM medium containing 8 μg/ml puromycin (Sangon Biotech, A610593-0025). A stable cell population overexpressing CD19 (hereafter referred to as the cCD19 cell population) was obtained by replacing the fresh drug medium with the fourth to fifth passage of each generation.

2.4.3. Western blot analysis of cCD19 cell protein

293T cells and cCD19 cells were lysed for 1 h on ice and centrifuged at 18,514×*g* for 20 min using a whole protein extraction kit (Solarbio, BC3710). The supernatant was boiled in SDS-PAGE Loading Buffer (Solarbio, P1040) and subjected to SDS-PAGE. After blocking for 2 h with a blocking solution containing 2 % bovine serum albumin (BSA), Mouse anti-His-Tag monoclonal antibody (AE003, ABclonal) was used as the primary antibody (1:2000) and incubated in 2 % BSA at 4 °C overnight. The following day, the PVDF membrane was washed 5 times with PBST and incubated in Goat Anti-Mouse IgG (H + L) (1:100,000) (Jackson, 115-035-003) in 2 % BSA at room temperature for 1 h. After 5 washes with PBST, the color was developed using an ultra-high sensitive ECL chemiluminescence kit (Beyotime Biotechnology, P0018AS) and exposed for 0–15 s to obtain the Western blot results.

2.5. Preparation of CD19 mAb

Monoclonal antibodies were generated following the guidelines of the Committee for the Control and Supervision of Animal Experiments (CPCSEA), with approval from the Animal Ethics Committee for the experimental protocol. Five SPF female BALB/c mice, aged 4–6 weeks, underwent immunization with 200 μg of CD19 peptide conjugated with KLH as an immunogen, emulsified 1:1 with complete Freund's adjuvant. The same CD19 peptide dose was emulsified 1:1 with incomplete Freund's adjuvant (Sigma, F5881-10 ml) every 2 weeks, totaling four times. Two weeks after each immunization, blood was collected from the mice's tail veins, and serum was separated by centrifugation. The serum antibody titer was determined through indirect ELISA.Based on the serum titer results, mice with the highest titers were selected for subcutaneous injection of 50 μg of CD19 polypeptide without adjuvant on the back for shock immunization. Cell fusion was performed 3 days after shock immunization, fusing splenocytes from boosted immunized BALB/c mice with SP2/0 cells using PEG4000 (Sigma, 25322-68-3). Hybridoma cells with successful fusion were screened in RPMI 1640 complete medium containing 1 % aminopterin (Sigma, A5159-10VL). After 7 days of screening, 100 μl of fusion cell culture supernatant was collected from each well for detection. Positive monoclonal wells with good cell morphology, high OD450nm values, and appropriate cell clone sizes were selected for limited dilution and subcloning culture, performed four times in total. Positive subclone strains were then screened based on ELISA test results.

2.6. CD19 mAb purification

In this study, two methods were employed for monoclonal antibody (mAb) preparation to purify ascites and cell supernatant. The ascites preparation involved selecting multiparous BALB/c mice, with each mouse receiving an intraperitoneal injection of 500 μl liquid paraffin one week prior to ascites collection. Ascites were then collected using a 2 ml disposable sterile syringe and centrifuged at 12,857×*g*/min at 4 °C for 10 min to obtain the supernatant. Approximately 3 ml of ascites were collected from each mouse.For the mAb preparation from cell supernatant, the culture medium supernatant was collected when the hybridoma cells reached 80%–90 % confluence, with 50 ml of culture medium supernatant collected for each hybridoma cell. Both the obtained ascites and cell supernatant were subsequently purified using a Protein G purification column (Zhongke Senhui, 15001006) to obtain monoclonal antibodies. The purity of the purified mAbs was then assessed through SDS-PAGE electrophoresis. The subtypes were identified using the Immediate-Use Mouse Monoclonal Antibody Ig Class/Subclass/Subtype Identification Kit (6 kinds) (Frabio, FRD90100P6Rd).

2.7. Affinity identification of CD19 mAb

2.7.1. ELISA detected antibody affinity

The microplate was coated with 2.5 μg/ml CD19 naked peptide unconjugated with KLH, 100 μl per well, and left to coat overnight at 4 °C. The next day, the plates were washed, and a blocking step was performed using 2 % BSA at 37 °C for 2 h. After washing the plates again, they were utilized as CD19 titer detection plates.The obtained CD19 monoclonal antibody (mAb) was diluted in different ratios as the primary antibody and added to the detection plate, 10 μl per well, and incubated at 37 °C for 1 h. Following another round of plate washing, secondary antibodies of Goat Anti-Mouse IgG were added to each well with 10 μl and incubated for 1 h at 37 °C. After washing the plate, TMB was added for the color reaction, and the absorbance at 450 nm wavelength was measured after terminating the reaction with sulfuric acid.

2.7.2. Antibody affinity was detected by Western blot

Proteins from 293T and cCD19 cells were extracted, with CD19 monoclonal antibody (mAb) serving as the primary antibody, and Goat Anti-Mouse IgG employed as the secondary antibody. Western blot analysis was conducted to obtain immunoblots depicting CD19 protein expression, and the protein expression levels in each group were quantified through grayscale analysis.

2.7.3. Antibody affinity was detected by flow cytometry

The concentration of 293T and cCD19 cells was adjusted to 1 × 10^6 viable cells/ml, and 1 ml of each cell was aliquoted into flow cytometry tubes. Following centrifugation at 417×*g* for 4 min, the cells were resuspended in 1 ml PBS, washed once, and the supernatant was discarded. After resuspension and two washes with 1 ml PBS, 40 μl of Alexa Fluor® 647 AffiniPure Donkey Anti-Mouse IgG (H + L) (1:400) (Jackson ImmunoResearch, 715-605-151) was added, incubated at 4 °C for 30 min, and then resuspended and washed twice with 1 ml PBS. Subsequent to washing, the cells were resuspended in 600 μl PBS, collected after filtration through a 70 μm cell screen, placed on ice, and analyzed using the Beckman Coulter Cytoflex.

2.7.4. Antibody affinity was detected by immunofluorescence

5 ml cat blood was collected and PBMCs were extracted from peripheral blood lymphocyte. The adhesive was diluted with PBS1:250 and the slides were immersed in the diluted solution for 30 min at 37 °C, occasionally shaken, then removed and dried. PBMCs were collected in a centrifuge tube, and then resuspended with 500 μl 4 % paraformaldehyde for 20 min at room temperature. The fixatives were washed by centrifugation with PBS, three times, each time for 3 min, and the last time, 50 mL of liquid was kept and resuspended. The cell suspension was added to the cell slides to make the cells evenly distributed and slightly dried. Subsequently, 0.2 % tritonx-100 was perforated for 15 min at room temperature and the cells were washed three times by PBS. After blocking the goat serum for 1 h, the antibody to CD19 was diluted at a ratio of 1:20. After adding the antibody, the antibody was washed three times in

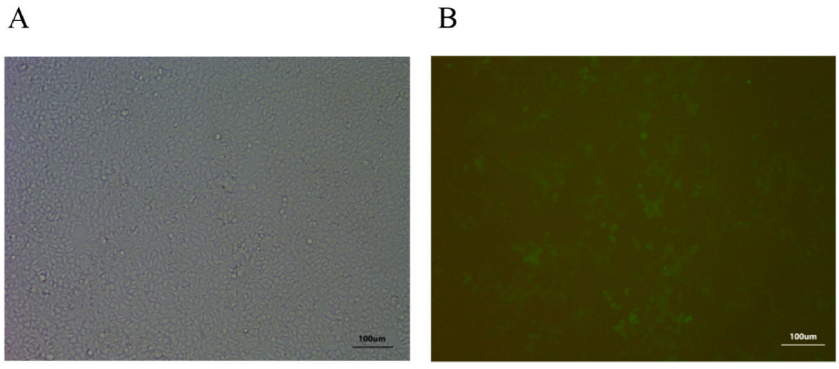

Fig. 2. Transfection efficiency analysis by fluorescence microscope. (A) field map of cCD19 cell; (B) Fluorescence image of cCD19 cells. Light field cell growth confluence of more than 90 %, fluorescence positive rate of more than 50 %, scale: 100 μm.

PBS at 4 °C overnight. Add second antibody, keep away from light, incubate at room temperature for 1h, wash PBS three times, then take photos.

2.8. Statistical analysis

The test data were presented as mean ± standard deviation (SD). One-way analysis of variance was conducted using GraphPad Prism 8 software, and intergroup differences were assessed by *t*-test. A significance level of 0.05 was considered statistically significant.

3. Result

3.1. Acquisition and identification of cCD19 cell line

293T cells were infected with the packaged CD19-OE lentivirus and underwent screening with 8 μg/ml puromycin for four generations to obtain fluorescent-labeled cCD19 cell populations. As depicted in Fig. 2(A,B), the positive transfection rate exceeded 50 %.

To further characterize the obtained cCD19 cells, we extracted proteins from both 293T and cCD19 cell populations and conducted Western blot analysis using a His-tag monoclonal antibody. The results revealed the absence of a specific protein band in 293T cells, while a distinct 70–100 kDa protein band was observed in cCD19 cells. Additionally, after incubation with β-actin, bands of 40–50 kDa were evident in both cell groups, consistent with the expected band size, indicating the successful expression of cCD19 (Fig. 3A and B).

3.2. Screening of positive hybridoma clones and purification and identification of mAbs

Spleen cells from immunized mice were fused with SP2/0 cells. Seven days post-fusion, initial screening was performed using indirect ELISA. Hybridoma cells in two wells exhibiting supernatant OD450nm values over 0.7 were selected for limited dilution subcloning. Following four subclone screenings, two positive monoclonal hybridomas were obtained and named 3H9 and 8A7 after their original wells. The two cell lines were injected into the peritoneal cavity of mice to obtain ascites. Simultaneously, cell culture supernatant and ascites were purified to obtain monoclonal antibodies (mAb).To confirm the purity of the purified CD19-3H9 and CD19-8A7 mAb, SDS-PAGE analysis was conducted. The results revealed two bands at 50 kDa and 25 kDa, consistent with the expected sizes of the antibody's light and heavy chains. This indicated that the antibody possessed a complete light and heavy chain structure, as illustrated in Fig. 4. The obtained mAbs were further subtyped using the Immediate-Use Mouse Monoclonal Antibody Ig Class/Subclass/Subtype Identification Kit (6 kinds). As shown in Table 2, the subtype of CD19-3H9 and CD19-8A7 ascites/supernatant purified mAb was identified as IgG1.

3.3. Affinity detection of CD19 mAbs

To assess the antibody affinity, an indirect ELISA was employed to determine the titers of purified monoclonal antibodies (mAbs) obtained from both ascites and cell supernatant through the Protein G purification column. The results indicated that the 3H9/8A7 mAb titers were not less than 1:32,000, with the ascites purification group achieving a titer of up to 1:128,000, as illustrated in Fig. 5A, B.

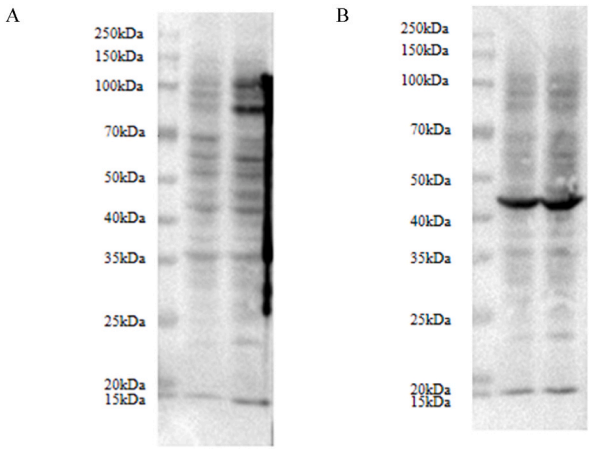

Fig. 3. cCD19 protein detection and analysis by Western blot. (A) results of HIS protein band; (B)results of β-actin protein band. The expression of cCD19 protein was detected by Western blot analysis. SDS-PAGE separated 293T cell proteins/cCD19 cell proteins, transferred to nitrocellulose membranes, and then combined with His-tag mAb (as a primary antibody), and Goat Anti-Mouse IgG (1:100000, as a secondary antibody) incubation, the values on the left indicate the approximate size of the protein band in kilodaltons. The uncropped images of Figure3(A,B) were referred in Supplementary Figure1.

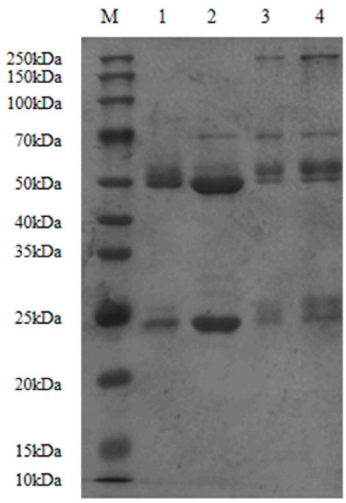

Fig. 4. The SDS-PAGE analysis of the chromatographic isolation of the mAb obtained. 1: Protein Maker; 2:3H9 mAb -from ascites; 3: 3H9 mAb -from supernatant; 4: 8A7 mAb -from ascites; 5: 8A7 mAb -from supernatant. Pure CD19-3H9 and CD19-8A7 mabs were separated by SDS-PAGE to confirm their heavy and light chain structures. M-lane is a protein molecular weight marker. The uncropped images of Figure4 were referred in Supplementary Figure2.

Table 2
ELISA titers for isotyping.

Clone	Subtype					
	IgA	IgM	IgG1	IgG2a	IgG2b	IgG3
3H9-from ascites	0.039	0.039	0.511	0.039	0.070	0.039
3H9-from supernatant	0.039	0.038	0.385	0.039	0.039	0.039
8A7-from ascites	0.040	0.039	0.741	0.039	0.040	0.039
8A7-from supernatant	0.040	0.039	0.566	0.038	0.039	0.040

3.4. Specific detection of CD19 mAbs

The specificity of the purified mAbs was identified by Western blot with 293T and cCD19 cell proteins. The results showed that the purified mAb CD19-3H9 and CD19-8A7 could specifically react with 293T and cCD19 cell proteins(Fig. 6A). According to the grayscale analysis, the gray value of CD19-3H9 (ascites/supernatant) was 0.84 and 0.78, and that of CD19-8A7 (ascites/supernatant) was 0.88 and 0.73, which were higher than those of 293T group. The protein affinity results of each antibody group are shown in Fig. 6B. The results showed that the affinity of each mAb group with cCD19 protein was significantly higher than that of 293T protein ($P < 0.05$).

3.5. Antibody affinity was detected by flow cytometry

Peripheral blood PBMCs from cats were stained with CD19-3H9/CD19-8A7 (from ascites/from supernatant) purified mAb as primary antibody and Alexa Fluor® 647 AffiniPure Donkey Anti-Mouse IgG (H + L) as secondary antibody. Flow cytometry showed that: Each mAb could specifically recognize some cells in PBMCs, and the cell population was apparent. The percentage of lymphocytes in gate 2 of each group was 25.9 %, 21.9 %, 24.1 % and 22.1 %, respectively, which was in line with the expectation. The results were shown in Fig. 7 (A,B), indicating that each mAb could specifically recognize the CD19-expressing cell population in cat peripheral blood PBMCs.

3.6. Antibody affinity was detected by immunofluorescence

Peripheral blood PBMCs from cats were immunofluorescent stained with mAb purified from CD19-3H9/CD19-8A7(from ascites/supernatant) as primary antibody and Goat Anti-Mouse IgG (h + L) FITC as secondary antibody. The results showed that CD19 mcab could specifically recognize lymphocyte in PBMCs with high specificity(Fig. 8A).

4. Discussion

Monoclonal antibodies have great application value in biological tests, medical research, and animal breeding [17,24,25]. Many commercialized mAbs have been widely used in the diagnosis and clinical treatment of tumor diseases [16], among which mAbs of

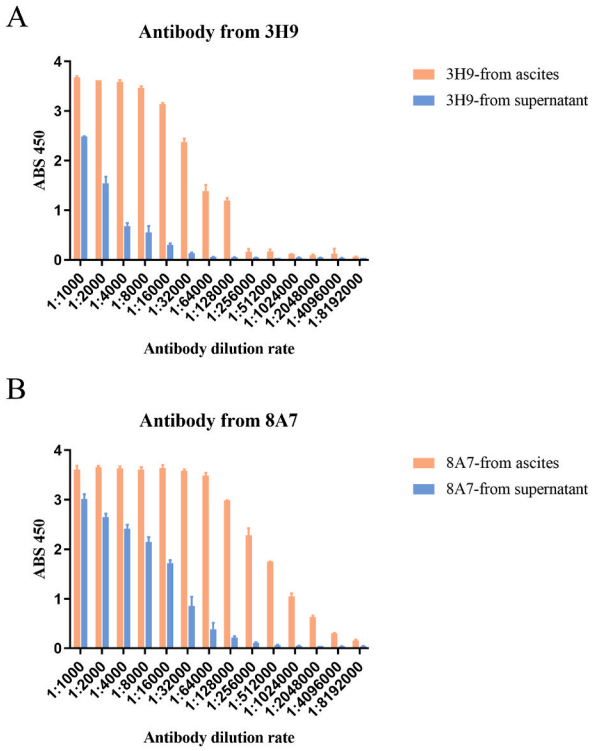

Fig. 5. The titer of purified mAb tested by ELISA. (A) 3H9 monoclonal antibody titer; (B) 8A7 monoclonal antibody titer. 3H9 mAb/8A7 mAb OD 450 nm values were measured using indirect ELISA; error bars indicate their SD.

B-cell surface marker molecules are one of the essential tools for the diagnosis and treatment of immune-related diseases [26]. As a central regulator regulating various signaling pathways in B cells, CD19 has a particular impact on the balance between humoral immune response and tolerance induction [27]. Therefore, the level of CD19 molecule can not only be used as a detection standard of B cell malignant tumor diseases and an ideal target for immune therapy [28,29]. In recent years, researchers have found that preclinical mouse models of many human diseases cannot accurately predict the safety or efficacy of treatment [30], so the establishment of dog and cat models with immune systems more similar to those of humans has become particularly important [31]. Canine and cat antibodies are not only essential research tools for establishing animal models but also essential for detecting and treating clinical diseases in dogs and cats. However, there are few reports on the research of canine and cat tumor antibodies at home and abroad, especially the preparation and development of antibodies for detecting and treating cat B-cell tumor diseases. Therefore, this study optimized and innovated the existing antibody preparation method and developed and verified a mouse anti-cat CD19 mAb, which can be applied to diagnosing and treating cat B-cell-related diseases. It also lays a foundation for establishing animal models for studying related diseases in humans, dogs, and cats.

In this study, peptides were designed and prepared by searching the structure-activity region of the cat CD19 protein peptide chain. Two hybridoma clones against cat CD19 protein were obtained using KLH-conjugated CD19 peptide as antigen. According to the traditional indirect ELISA screening method, two hybridomas were screened by limiting dilution for four generations to obtain monoclonal cell lines 3H9 and 8A7. Based on the traditional method of mAb preparation from ascites, we purified the cell culture supernatant to prepare mAb and then performed the affinity test. In order to further identify the mAb obtained, the cat CD19 overexpression vector was constructed and prepared and transfected into 293T cells. The CD19 overexpression cell population was screened by puromycin, and the overexpression of CD19 was verified by fluorescence microscopy and Western blot. The results showed that the CD19-expressing cell population was successfully screened and expressed. Subsequently, the screened mAbs were identified by ELISA, Western blot, and flow cytometry to determine the immunological detection effect of the obtained mAbs. The results of ELISA and Western blot showed that 3H9 and 8A7 mAbs could bind to CD19 polypeptide sequence and eukaryotic expressed protein. At the same time, CD19 mAb prepared in this study could be used to group lymphocytes in PBMCs of dogs and cats by flow cytometry, and the grouping was specific. In peripheral blood, CD19 is mainly expressed on the surface of B cells [32], and the proportion of $CD19^{+}$B cells should be 14–21 % [26]. In this study, the proportion of B cells in cat PBMCs recognized by 3H9-mAb and 8A7-mAb was within the expected range. The mAb prepared in this study could specifically recognize the CD19 epitope of B cells in the peripheral blood of cats.

The traditional mAb preparation method mainly adopts the method of mouse ascites preparation, which can obtain a large number of mAb with high purity [33]. However, it has the characteristics of complex operation and long preparation cycles, so it is not thoroughly tested in mAb development and small-scale testing. Therefore, we aim to optimize a more simple and efficient purification and preparation method based on the existing mAb preparation method. We found that in the traditional hybridoma cell screening

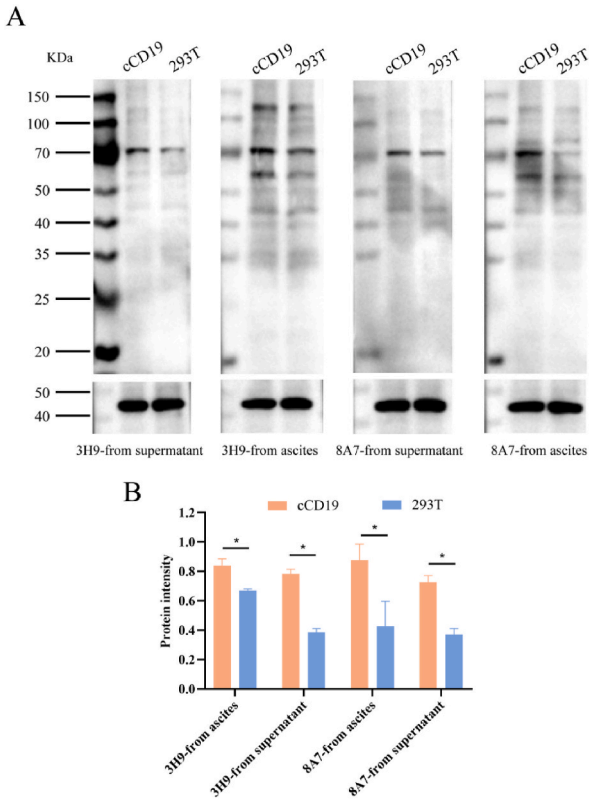

Fig. 6. (A) Western blotting was performed to detect cCD19 expression in CD19 overexpressing cells as well as negative control 293T. (B) Quantitative analysis of CD19 and 293T expression levels. All data are mean ± SD (n = 3), *p < 0.05, **p < 0.01, ***p < 0.001. The uncropped images of Figure6A were referred in Supplementary Figure3.

process, the OD value of the cell supernatant was used as the evaluation standard [34], which proved that in the process of hybridoma culture, the cell culture supernatant contained the target antibody. The small amount of mAb in the cell culture supernatant was insufficient to achieve the detection concentration. Therefore, in this study, a large number of cell culture supernatants collected were purified by the Protein G column, and the affinity of the purified mAb was identified. According to the comparison of the results of the mAb application test, we can find that the mAb prepared by using ascites and cell culture supernatant have a specific affinity for CD19 molecule, which proved that the optimized mAb preparation method of cell culture supernatant in this study could meet the requirements of the target mAb preparation and detection. Analysis of the mAb detection results showed that both CD19-3H9 and CD19-8A7 mAb could specifically recognize CD19 structure activity region peptide, CD19 eukaryotic cell expression site, and $CD19^{+}$ PBMCs. A comprehensive recognition anchoring of mAb to cat CD19 protein molecules from linear to spatial structure was achieved.

In summary, this study optimized a rapid and straightforward monoclonal antibody preparation method and designed and prepared cat CD19 monoclonal antibody, which can be used as an essential tool for the identification and research of cat CD19 functional sequence and application value, to a certain extent, to fill the gap in the research field and commercial market of cat CD19 molecular monoclonal antibodies.

5. Conclusion

In this study, cat CD19 conjugated peptides were synthesized to immunize mice, and monoclonal hybridoma cell lines were obtained. The existing mAb preparation method was optimized, and the affinity of the mAb was detected by the constructed and screened eukaryotic overexpression cell population, and the cat CD19-specific monoclonal antibody was successfully obtained. The monoclonal antibody prepared in this study is suitable for ELISA, Western blot, Immunofluorescence and flow cytometry detection and lays a foundation for targeted immunotherapy for B-cell tumor disease detection and the establishment of large animal models.

Ethical statement

The experimental protocol was approved by the Animal Ethical and Welfare Committee(AEWC) and was performed in accordance with animal care and Ethical guidelines. Animal Ethics Committee approval number was IRM-DWLL-2022242.

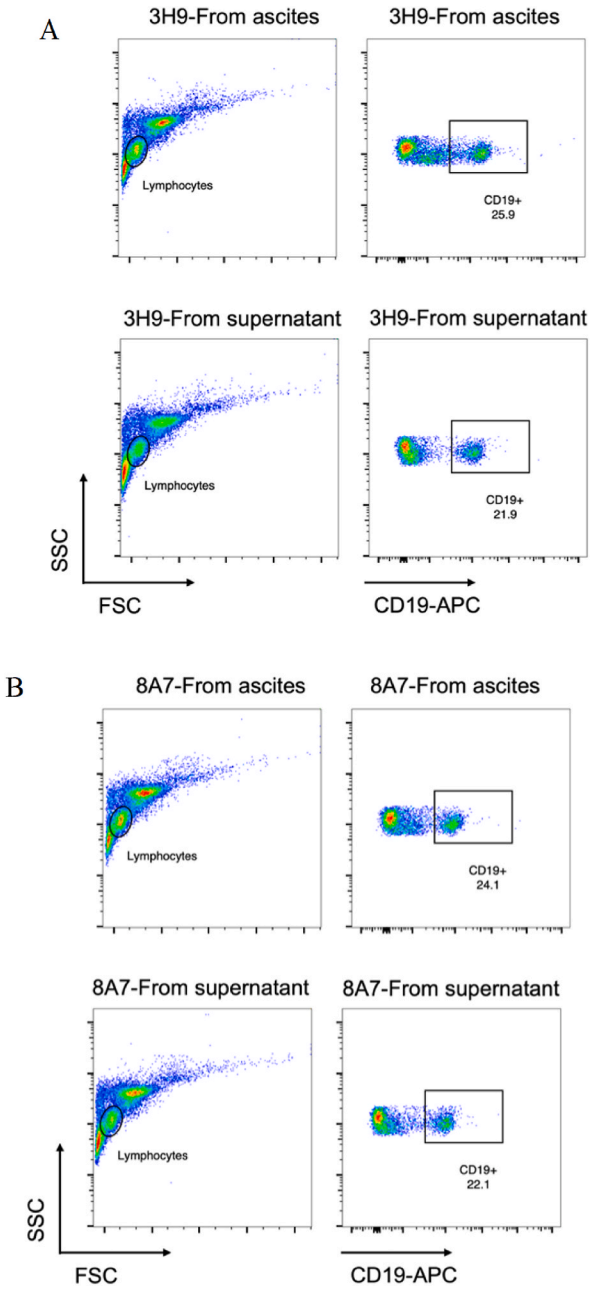

Fig. 7. Flow cytometric analysis of CD19 mAb. (A) flow cytometric analysis of 3H9-from ascites/supernatant; (B) flow cytometric analysis of 8A7-from ascites/supernatant. CD19 mAb in peripheral blood mononuclear cells (PBMCs) was detected by flow cytometry. CD19-3H9/CD19-8A7 mAb both bound to $CD19^{+}$ B cells in PBMCs.

Funding information

This work was supported by the Nourse Science Centre for Pet Nutrition (petABCD202105).

Data availability statement

Source data were included in article and supplementary materials, and the data associated with this study have not been deposited in a publicly available repository.

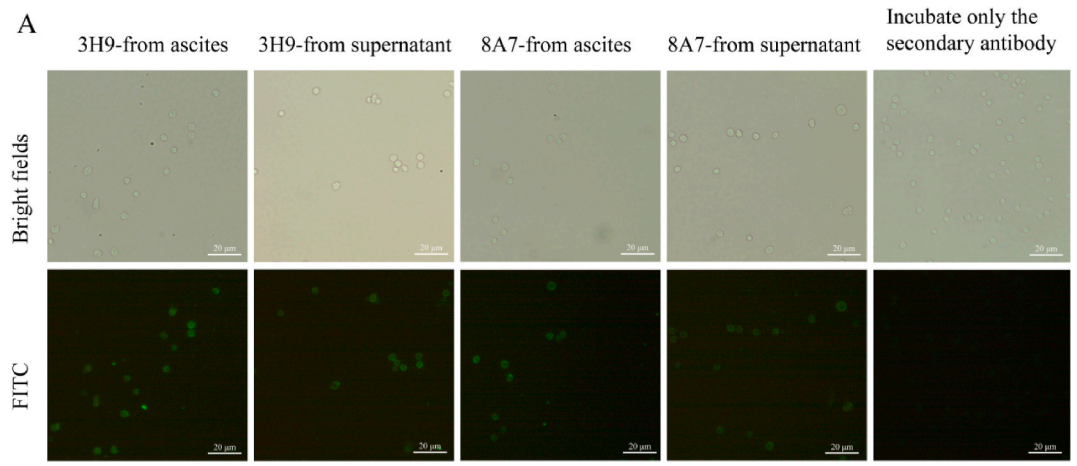

Fig. 8. (A) Immunofluorescent staining of CD19 expression in PBMCs. All images had the same scale of 20 μm. The uncropped images of Figure8A were referred in Supplementary Figure4.

CRediT authorship contribution statement

Liya Shen: Writing – review & editing, Investigation. **Shuqi Yan:** Investigation, Data curation. **Aoyu Xu:** Writing – review & editing, Writing – original draft, Investigation. **Di Lan:** Writing – original draft, Investigation. **Xue Jiang:** Writing – review & editing, Writing – original draft, Investigation, Data curation. **Yuehan Peng:** Investigation, Writing – original draft. **Songjun Wang:** Investigation. **Zhanzhong Wang:** Methodology. **Yongyan Chen:** Writing – review & editing, Writing – original draft, Visualization, Supervision.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Liya Shen, Shuqi Yan, Xue Jiang, Yuehan Peng, Di Lan and Songjun Wang were all employees of Nourse Science Centre for Pet Nutrition, Shanghai Chowsing Pet Products Co., Ltd, Wuhu Weishi Biotechnology Co., Ltd during the study period. These units involve pet nutrition products, pet medical basic research commodities and other products, as well as public welfare resources such as dog and cat cell lines. Our research may contribute to the development of these areas. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank PETCC, the Global Pets' Cell Resource Center, for kindly providing us with cell lines and medium. We thank Ruxin Zhao for testing methods of cat CD19 monoclonal antibody preparation and assistance during the manuscript revision.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e33145.

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