A pair of SARS-CoV-2 nucleocapsid protein monoclonal antibodies shows high specificity and sensitivity for diagnosis

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Dear Editor,

Severe Acute Respiratory Syndrome Coronavirus Type 2 (SARS-CoV-2) is the pathogen of the coronavirus disease 2019 (COVID-19), which has spread worldwide. SARS-CoV-2 is an enveloped virus belongs to the family of β-coronavirus (Xu et al., 2020). Its genome is a linear single-stranded positive-sense RNA about 30,000 nucleotides packed inside a virion with a diameter of 100 nm and a volume of about 10^6 nm^3 (Bar-On et al., 2020). The genome of SARS-CoV-2 encodes 29 proteins, including 16 nonstructural, four structural, and nine accessory proteins (Bai et al., 2021). The four structural proteins are spike (S) glycoprotein, envelope (E) protein, membrane (M) protein, and nucleocapsid (N) protein (Naqvi et al., 2020), which are shared with other coronaviruses (Brian and Baric, 2020; Zarbock, 2020). Accordingly, multiple strains of SARS-CoV-2 have emerged, such as Alpha, Beta, Gamma, Delta, and Omicron, etc.

The nucleocapsid (N) protein consists of three domains: an amino-terminal RNA-binding domain (NTD), a carboxyl-terminal dimerization domain (CTD), and an intrinsically disordered central Ser/Arg (SR)-rich linker (Wang et al., 2022). The N protein is highly immunogenic and abundant in serum of infected patient (Burbeilo et al., 2020). It is thus a good serological antigen for diagnosis of SARS-CoV-2 infection (Nii et al., 2020; Xiang et al., 2020; Zeng et al., 2020). SARS-CoV-2 is highly contagious and spreads very quickly, thus it is urgent to develop an accurate diagnosis. However, most of the effects nowadays are devoted to produce blocking antibody through screening B cells from the patients (Cohen-Dvashi et al., 2022; Ju et al., 2020; Sun et al., 2022) while fewer reports on producing antibodies for diagnosis (Wang et al., 2022). In this study, we aimed to produce monoclonal antibody (mAb) against the N protein of SARS-CoV-2, which we hope will be useful for COVID-19 diagnosis.

First, the N protein was cloned into the restriction enzyme sites Nco I and Sal I of PET-28a (+) to generate the pET-28a (+)-SARS-CoV-2 (N) recombinant plasmid (Supplementary Fig. S1A), which was then transformed into E. coli Rosetta (DE3). Expression of the N protein was induced with IPTG (Isopropyl-β-D-thiogalactopyranoside) at the concentration of 1 μmol/L, and the induced N protein was further purified with a Ni-NTA affinity chromatography column (QIAGEN, Cat#30210, Valencia, CA, USA) (Fig. 1A). The purified protein was injected into three BALB/c mice to produce mAb according to a standard procedure (Supplementary Table S1). After immunization, seven hybridoma cell lines stably secreting mAbs against N protein were obtained and named as 1C7, 1D5, 2E11, 2G11, 3C6, 4F10, and 5E11, respectively. Karyotyping confirmed that these mAbs were hybridoma cells (Fig. 1B). The isotypes of these mAbs were determined using a kit from Proteintech (PK 20002, Wuhan, China) (Supplementary Table S2).

We then determined whether these mAbs could be applied for Western blotting (WB). The N protein was cloned into pcDNA 3.1 (+) to generate the pcDNA 3.1 (+)-SARS-CoV-2 (N) expression plasmid (Supplementary Fig. S1B). Cell lysates (50 μg/lane) from 293T cells transfected with pcDNA 3.1 (+)-SARS-CoV-2 (N) plasmid were...
separated by a 12 % SDS-PAGE and transferred onto a nitrocellulose membrane, and cell lysates transfected with the vector pCDNA 3.1 (+) plasmid were used as the negative control. The purified mAbs were used to detect the expression of N protein with a concentration of 1 ng/μl, and mouse IgG1 at the same concentration was used as a negative control. Polyclonal antibody against hemagglutinin (HA) was used as a positive control to identify the molecular weight of the expressed protein (Tianjin Sungee Biotech, Cat#K8M004, Tianjin, China) (Fig. 1C). WB assay showed that all produced mAbs reacted specifically against the N protein of SARS-CoV-2. However, 3C6 showed the weakest reaction while 2G11 is the strongest (Fig. 1C). In addition, 2G11 also identified a protein with smaller molecular weight (Fig. 1C), which is most likely a proteolytic product of the N protein since it was not detected in the vector transfected cells. The seven purified mAbs were further assessed in immunofluorescence (IF) staining assay with a concentration of 10 ng/μl and mouse IgG1 at the same concentration as the negative control. We found that all mAbs showed cytoplasmic staining although 2E11, 4F10, 2G11, and 5E11 had occasional nuclei staining (Fig. 1D).

To investigate which domain of N protein was recognized by the mAbs, we cloned the amino-terminal domain (1–173), the central domain (130–291), and the carboxyl-terminal domain (263–419) of SARS-CoV-2 N protein into pGEX-6p-1, respectively (Supplementary Fig. S1C). Primers used for expressing recombinant SARS-CoV-2 N protein were listed in Supplementary Table S3. The SDS-PAGE assay confirmed that all truncated N proteins were successfully expressed (Fig. 1E). Then in the enzyme linked immunosorbent assay (ELISA), the three truncated and the full-length N proteins were used to determine which domain of SARS-CoV-2 N protein was recognized by the mAbs. It turned out that the mAbs (2G11 and 1D5) recognized the amino-terminus of SARS-CoV-2 N protein and the full-length N protein (Fig. 1F). Another four mAbs (1C7, 2E11, 3C6, and 4F10) could only recognize the carboxyl-terminal domain of SARS-CoV-2 N protein and the full-length N protein (Fig. 1F). In contrast, we could not identify which domain of N protein was recognized by mAb 5E11 (result not shown), and the exact reason remains unknown. Since 1C7 and 2G11 seem to recognize distinct domains of N protein with relatively high affinity, we further investigated if these two mAbs could react to the two domains of N protein via WB. The results showed that 2G11 recognized the amino-terminus while 1C7 only recognized the carboxyl-terminal domain of SARS-CoV-2 N protein. As expected, mouse IgG1 used at the same concentration did not show any positive reaction while a mAb specific against GST Tag identified a positive signal with expected molecular weight in WB assay (Fig. 1G).

We then determined whether these mAbs could differentiate N proteins of different coronaviruses with ELISA assay. Thus, the seven mAbs were added into ELISA plate coated with different N proteins (20 ng/well) from MERS-CoV, Human Coronavirus (HCoV)-229E, SARS-CoV-1 and SARS-CoV-2 in an ELISA assay. The result showed that these mAbs did not cross react with the N protein of MERS-CoV and HCoV-229E but blocked with 3% BSA/PBST. Then 50 ng/well SARS-CoV-2 N protein was loaded. Human plasma (1 μl/well) from eight healthy donors (Sample No. 1–8) and 31 SARS-CoV-2 infected patients (Sample No. 9–47) were then loaded. Sample numbers underlined mean the samples from the same patient but at different dates. Bound human IgG was detected with preabsorbed HRP conjugated goat anti-human IgG. Sample information is listed in Supplementary Table S4. Full experiment details are given in the Supplementary file.
Here, we report the production of seven mAbs against SARS-CoV-2 N protein. These mAbs can be used successfully for WB and IF assay, and most of them can also be applied in ELISA. Although these mAbs cannot differentiate N protein of SARS-CoV-1 from that of SARS-CoV-2, they cross reacted to N protein of neither MERS-CoV nor HCoV-229E. It is worth noting that the 2G11/bio-1C7 mAbs pair can detect SARS-CoV-2 N protein as low as 15 pg/well. This sensitivity is comparable to that of an ELISA kit available. In addition, we developed an ELISA method to detect human plasma IgG against SARS-CoV-2 N protein, which showed a sensitivity of 97.4% and a specificity of 100%. Although we have identified approximate domains recognized by these mAbs, the exact epitopes reacted with these mAbs remain investigated. In a word, we produced several mAbs against the N protein of SARS-CoV-2, among which shows high specificity and sensitivity for SARS-CoV-2 diagnosis.

Footnotes

This study was conducted according to ARRIVE guidelines and all animal experimental procedures approved by the Institutional Animal Care and Use Committee of the First Affiliated Hospital of Guangzhou Medical University and State Key Laboratory of Respiratory Diseases, China (Protocol Permit Number: 2021243 and 2021338; approved on July 2021 and August 2021). Mice were maintained in Specific Pathogen Free (SPF) animal facility of Laboratory Animal Center, Guangzhou Medical University. The authors declare that they have no conflict of interest.

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