JOURNAL OF CLINICAL MICROBIOLOGY, May 2004, p. 2351–2352 Vol. 42, No. 5
0095-1137/04/$08.00+0 DOI: 10.1128/JCM.42.5.2351–2352.2004
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Antigenic Cross-Reactivity between the Nucleocapsid Protein of Severe Acute Respiratory Syndrome (SARS) Coronavirus and Polyclonal Antisera of Antigenic Group I Animal Coronaviruses: Implication for SARS Diagnosis

Severe acute respiratory syndrome (SARS) is an emerging infectious disease of significant public health concern (3). The causative agent of SARS was shown to be a previously unrecognized virus within the family *Coronaviridae*, designated SARS-associated coronavirus (SARS-CoV) (1, 4, 7). There exist three known antigenic groups (I, II, and III) of animal coronaviruses, causing important and severe respiratory and enteric diseases in livestock, poultry, and laboratory animals and common colds (strains 229E and OC43) in humans (3). Sequence analyses revealed that SARS-CoV is not a derivative of any known animal coronaviruses (5, 8). Nevertheless, Ksiazek et al. (4) showed that polyclonal antibodies from antigenic group I coronaviruses, including human coronavirus 229E, feline infectious peritonitis virus (FIPV), and porcine transmissible gastroenteritis virus (TGEV), reacted with SARS-CoV-infected Vero cells.

Since the nucleocapsid (N) proteins of known coronaviruses are relatively conserved, we aimed to determine if the N protein is responsible for the observed antigenic cross-reactivity (4). The N gene of the SARS-CoV contains no glycosylation sites (5, 8), and thus we expressed and characterized the N protein in *Escherichia coli*. Briefly, the N gene was amplified by reverse transcription-PCR with a set of primers (forward primer 5′-CCCGGATCCAAATGTCTGATAATGGACCCC-3′ and reverse primer 5′-CCCGAATTCTTATGCCTGAGTTGAATCAGC-3′) containing engineered restriction enzyme (BamHI and EcoRI, respectively) sites (underlined). The amplified N gene was cloned in frame with the sequence coding for Xpress epitope located upstream of the multiple cloning site of the pRSET-C expression vector. The recombinant plasmids were transformed into *E. coli* strain BL21 Star (DE3)(pLysS) cells (Invitrogen), which produced T7 polymerase, and the expression of the fusion N protein was driven by a T7 promoter upstream of the Xpress epitope and induced by the addition of 1 mM IPTG (isopropyl-β-D-thiogalactopyranoside) (Fig. 1). The SARS-CoV N protein was purified with the BugBuster His-Bind purification kit (Novagen), based on the affinity of His-Bind resin for His-tagged fusion N protein, and confirmed with a monoclonal antibody to the fused Xpress epitope (Fig. 1) and with a convalescent-phase SARS patient serum (Fig. 2) by Western blot.

Western blot analysis was used to determine if the N protein of SARS-CoV cross-reacts with polyclonal antisera of known animal coronaviruses (Fig. 2). The purified N protein was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred on to a nitrocellulose membrane. After blocking, the membranes were incubated with a 1:100 dilution of a polyclonal antiserum to either human, bovine, swine, chicken, turkey, canine, or feline coronaviruses in Tris-buffered saline containing 0.05% Tween 20 (Fig. 2). After incubation with respective horseradish peroxidase-conjugated secondary antibodies at a 1:1,000 dilution, the immunocomplexes were detected with 4-chloro-1-naphthol. The results showed that the N protein of SARS-CoV reacted, as expected, with a convalescent-phase SARS patient serum, but it also reacted strongly with polyclonal antisera of known antigenic group I coronaviruses tested in the study including TGEV, FIPV, and canine coronavirus (CCoV), indicating that the N protein of SARS-CoV shares common antigenic epitope(s) with that of antigenic group I animal coronaviruses. The N protein of SARS-CoV, however, does not cross-react with polyclonal antisera from antigenic group II (porcine hemagglutinating encephalomyelitis virus [HEV] and bovine coronavirus [BCoV]) or group III (turkey coronavirus [TCoV] and avian infectious bronchitis virus [IBV]) animal coronaviruses tested in our study (Fig. 2).

The results from this study raised potential concerns for using recombinant N protein of SARS-CoV, whole-virus antigen extracts, or virus-infected cells as reagents for diagnosis of SARS-CoV infections in humans and other animal species (2, 4, 9). The antigenic group I coronaviruses are known to infect a variety of animal species, including swine, canines, felines, rabbits, and humans. Thus, the use of native SARS-CoV N protein or whole virus in enzyme-linked immunosorbent assay or indirect immunofluorescence assay (IFA) and the use of SARS convalescent-phase sera or polyclonal antibody raised against native SARS-CoV N protein or whole virus in direct IFA could produce a false-positive diagnosis of SARS, although this concern is very minimal since the two known human coronaviruses (strains 229E and OC43) do not cause severe clinical diseases. Although the natural animal reservoir for SARS-CoV has not yet been identified, it is believed that SARS-CoV originated from wild animal species (2, 6). Therefore, the use of N protein or whole virus as diagnostic antigens could also complicate the search for a definitive natural animal reservoir, as many wild and domestic animal species may have already been infected by known group I coronaviruses. There-

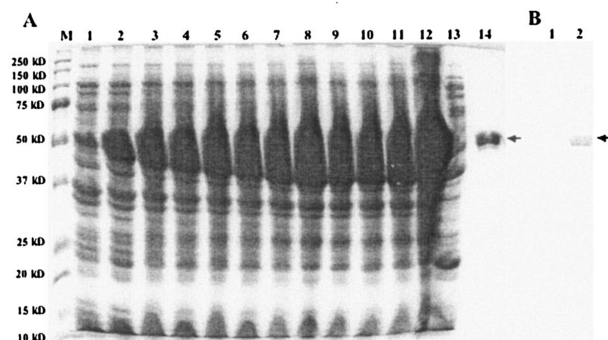

FIG. 1. (A) Expression of the N protein of SARS-CoV in *E. coli*. Lanes 1 to 12, SDS-PAGE of bacterial cell lysates harvested at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 18 h, respectively, after IPTG induction; lane 13, soluble proteins in the supernatant of cell lysates; lane 14, SDS-PAGE of 5 μg of the purified N protein. (B) Western blot of soluble proteins in the supernatant (B1) and of the purified protein (B2) using monoclonal antibody to the Xpress epitope tag fused with the N protein. A molecular marker (M) is indicated. Arrows, 50 kDa.

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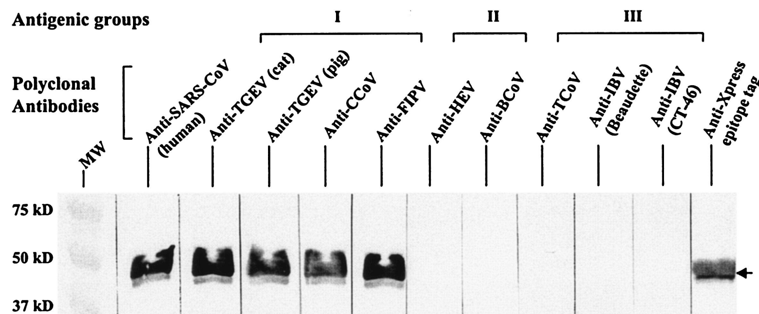

FIG. 2. Western blot analyses of antigenic cross-reactivity of SARS-CoV N protein with polyclonal antisera of known animal coronaviruses. SARS-CoV N protein (1 μg/lane) was separated by SDS-PAGE. Each antiserum was diluted 1:100 in blocking solution. A convalescent-phase SARS human patient serum (anti-SARS-CoV) and anti-Xpress antibody were used as positive controls. The polyclonal antisera used in the Western blot analysis were from antigenic group I (FIPV, porcine TGEV, and CCoV), group II (porcine HEV and BCoV), and group III (TCoV and avian IBV) animal coronaviruses. Polyclonal antisera to IBV, HEV, BcoV (calf serum), and TGEV (pig serum) were purchased from National Veterinary Service Laboratories, Ames, Iowa. Polyclonal cat antisera to TGEV and CCoV and cat ascitic fluid against FIPV were purchased from VMRD, Inc., Pullman, Wash. The arrow shows the expected size (about 50 kDa) of the SARS-CoV N protein.

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fore, it is important to identify a specific N protein immunoreactive epitope or other protein specific only for SARS-CoV with no antigenic cross-reactivity to known coronaviruses as the antigen for SARS diagnosis and for identification of the SASR-CoV animal reservoir(s).

We thank Dean Erdman, Paul Rota, and Thomas Ksiazek of the Centers for Disease Control and Prevention, Atlanta, Ga., for generously providing SARS-CoV RNA and SARS convalescent-phase patient serum and F. W. Pierson of Virginia Tech for providing TCoV antibody.

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