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Conserved amino acids W423 and N424 in receptor-binding domain of SARS-CoV are potential targets for therapeutic monoclonal antibody

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ABSTRACT

The receptor-binding domain (RBD) on spike protein of severe acute respiratory syndrome-associated coronavirus (SARS-CoV) is the main region interacting with the viral receptor-ACE2 and is a useful target for induction of neutralizing antibodies against SARS-CoV infection. Here we generated two monoclonal antibodies (mAbs), targeting RBD, with marked virus neutralizing activity. The mAbs recognize a new conformational epitope which consists of several discontinuous peptides (aa. 343–367, 373–390 and 411–428) and is spatially located neighboring the receptor-binding motif (RPM) region of the RBD. Importantly, W423 and N424 residues are essential for mAb recognition and are highly conserved among 107 different strains of SARS, indicating that the residues are the most critical in the epitope which is a novel potential target for therapeutic mAbs. A human–mouse chimeric antibody, based upon the original murine mAb, was also constructed and shown to possess good neutralizing activity and high affinity.

Introduction

Severe acute respiratory syndrome (SARS), which spread widely in 2002 to 2004 in China and all over the world, resulted in a great loss of life and property. This fatal disease was caused by a newly identified coronavirus: severe acute respiratory syndrome-associated coronavirus (SARS-CoV) (Rota et al., 2003). SARS-CoV is a new kind of coronavirus that is significantly different from previously known ones (Stadler et al., 2003). It is a single-stranded positive-sense RNA virus, which contains four structural proteins embedded on the surface of the viral particle (Stadler et al., 2003). Among them, it has been demonstrated that spike protein (S protein) is the important functional protein that mediates entry of the virus into susceptible cells. The S protein of SARS-CoV is a type I transmembrane glycoprotein that consists of 1255 amino acids. It has two functional domains: S1 (aa. 1–680) and S2 (aa. 681–1255) as predicted by sequence alignment with S proteins of other coronaviruses (Rota et al., 2003; Holmes, 2003).

The S1 domain mediates viral attachment to the cellular receptor ACE2 (Li et al., 2003). The functional portion within the S1 domain, which directly interacts with ACE2, has been identified as the receptor-binding domain (RBD) (Wong et al., 2004). It is located between aa. 318–510 and has been proven to be a major neutralizing determinant (Wong et al., 2004; He et al., 2004a). It has been demonstrated that RBD can induce highly potent neutralizing antibodies. Therefore, it can be considered an excellent candidate for development of a subunit vaccine against SARS (He et al., 2004a). Neutralizing antibodies targeting RBD can be

Abbreviations: SARS, severe acute respiratory syndrome; SARS-CoV, severe acute respiratory syndrome-associated coronavirus; mAb/pAb, monoclonal/polyclonal antibody; RBD, receptor-binding domain; RBD-Fc, receptor-binding domain linked with a human IgG Fc; RPM, receptor-binding motif; ACE2, angiotensin-converting enzyme 2; CPE, cytopathic effect; pfu, plague forming unit.

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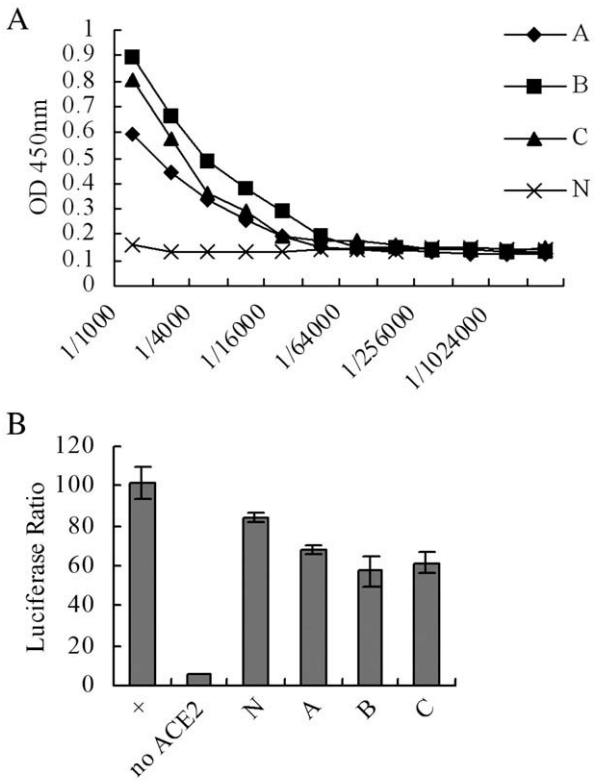

Fig. 1. RBD-specific neutralizing Ab was induced in mice. (A) A vaccinia virus which contains the full length of the spike gene was used to immunize mice. Titers of the RBD-specific Ab were tested using ELISA in sera collected 1 week after the second immunization. 'A', 'B' and 'C': three individual immunized mice, 'N': mouse without immunization. (B) Neutralizing activity was measured by syncytia inhibition assay. Immunized mouse sera collected 1 week after the second booster immunization was screened by syncytia inhibition assay. 'No ACE2': HEK293T cells without ACE2 plasmid transfection. '+': positive control in which HEK293T cells were transfected with ACE2 plasmid and no antiserum added. Neutralizing effects of normal mouse sera (N) and immunized mouse sera (A, B and C) were measured in groups of ACE2 transfected HEK293T cells. Mouse whose serum bears highest neutralizing activity was selected for raising mAbs.

generated by direct immunization of mice with inactivated SARS virus or RBD protein (He et al., 2004a, 2004b).

Identification of regions containing crucial amino acids within the RBD is essential for assuring recognition of neutralizing antibodies. Such identification could help to increase the understanding of the neutralizing mechanism and to aid discovery of potential drug targets.

So far, it has been reported that the RBD contains several linear and conformational epitopes, among which, most of the neutralizing epitopes are conformational ones (He et al., 2005). There are also some specific fragments or crucial amino acids within the RBD region that are related to neutralizing activity (Chakraborti et al., 2005; Yi et al., 2005). Recently the crystal structure of the RBD and ACE2 complex was clearly demonstrated and a receptor-binding motif (RPM aa. 424–494) within the RBD was characterized as the important region that directly contacts ACE2 (Li et al., 2005).

Although the studies mentioned above brought us much information, neutralizing epitopes have still not been completely characterized. In particular, detailed information concerning conformational neutralizing epitopes and critical amino acids is limited. Moreover, recent studies have shown that the RPM is an important region recognized by neutralizing monoclonal antibodies (Prabakaran et al., 2006; Hwang et al., 2006). However, information on neutralizing epitopes existing outside the RPM was still incomplete.

In this study, we generate two neutralizing monoclonal antibodies, termed S-9-11 and N-176-15, against the RBD. They exhibit good neutralizing activity in a syncytia inhibition assay, a pseudovirus infection assay and a neutralization assay in SARS virus infected cells. A mapping experiment indicates that both mAbs recognize several discontinuous peptides (aa. 343–367, 373–390 and 411–428) and that binding activity is conformation-dependent. Mutations of W423 and N424 completely abrogate binding activity of the RBD to the neutralizing monoclonal antibodies. It was further revealed that W423 and N424 residues within a 411–428 peptide are located adjoining the receptor-binding motif (RBM) of the RBD, an important region holding the RBD–ACE2 interaction surface. W423 and N424 are very stable among 107 different strains of SARS (Song et al., 2005), indicating that the mAbs would possibly possess a broad neutralizing spectrum. A human–mouse chimeric antibody was thus constructed based upon the N-176-15 mAb. It retained a high affinity ($KD = 1.6 \times 10^{-10}$ M) as compared to the original murine mAb ($KD = 2.8 \times 10^{-9}$ M). In addition, it maintained binding specificity and neutralizing activity.

The present work describes discovery of a new conformational epitope in the RBD located outside the RPM and formed by several discontinuous peptides with conserved amino acids W423 and N424 as critical mAb binding sites. These findings will be helpful in development of therapeutic antibodies against SARS-CoV.

Results

Vaccinia virus induces high titers of antibodies against RBD

In order to produce a useful antigen that mimics natural Spike protein on the particle surface of SARS-CoV, the full length S gene was

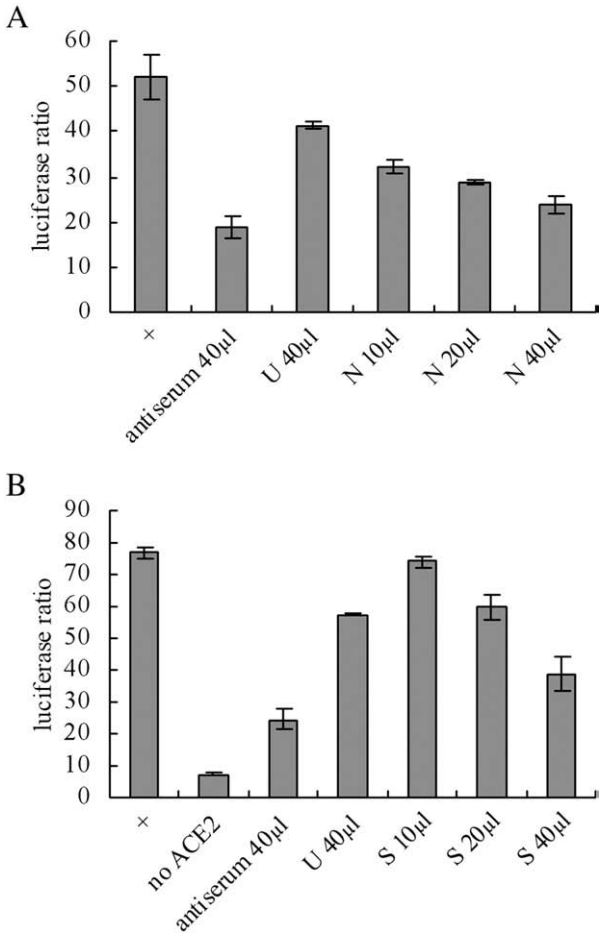

Fig. 2. Neutralizing activity of mAbs (A: N-176-15 and B: S-9-11) at different doses was determined by syncytia inhibition assay. The method is the same as that described in Fig. 1. '+': no mAb or antiserum. 'No ACE2': HEK293T cells without ACE2 plasmid transfection. 'U': An anti-mouse CD4 mAb selected as an unrelated control. 'S': mAb S-9-11, 'N': mAb N-176-15, the volume of mAb ascites added into each well (with medium of 1 ml) was also marked.