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Potent Human Monoclonal Antibodies against SARS CoV, Nipah and Hendra Viruses

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Abstract

Polyclonal antibodies have a century-old history of being effective against some viruses; recently, monoclonal antibodies (mAbs) have also shown success. The humanized mAb Synagis (palivizumab) remains still the only mAb against respiratory syncytial virus (RSV) infections approved by the U.S. Food and Drug Administration (FDA). Recently, several potent human monoclonal antibodies (hmAbs) targeting the Severe Acute Respiratory Syndrome-Associated coronavirus (SARS CoV) S glycoproteins were developed quickly after the virus was identified in 2003. Among these antibodies, m396 and S230.15 exhibit exceptional potency and cross-reactivity as they neutralize isolates from the first and second outbreaks and from palm civets both *in vitro* and in mice. Similarly, the first fully hmAbs against two other paramyxoviruses, Hendra virus (HeV) and Nipah virus (NiV), which can cause up to 75% mortality, were recently developed; one of them, m102.4, shows exceptional cross-reactive potency against both NiV and HeV. Three-dimensional molecular structures of envelope glycoproteins from these viruses in complexes with antibodies and/or receptors were recently determined. Structural analyses along with other experiments have provided insights into the molecular mechanisms of receptor recognition and antibody neutralization, and suggested that these antibodies alone or in combination could successfully fight the viruses' heterogeneity and mutability which is a major problem in the development of effective therapeutic agents against viruses, including therapeutic antibodies.

Keywords

antibody; SARS CoV; Hendra virus; Nipah virus; therapeutics; vaccines

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1. Introduction

Antibodies play an important role in recovery and protection from viral infections [1,2]. Sera from humans or animals have been widely used for prophylaxis and therapy of viral and bacterial diseases since the late 1800s [3–6]. Serum therapy of most bacterial infections was abandoned in the 1940s after antibiotics became widely available [5]. However, polyclonal antibody preparations have continued to be used for some toxin-mediated infectious diseases and venomous bites [3]. Serum immunoglobulin has continued to be also used for some viral diseases for which there are no other treatments available although mostly for prophylaxis either prior to an anticipated exposure or immediately following an exposure to an infectious agent [7–9]. Antibody products licensed in the US for prevention or treatment of viral diseases include non-immune human immunoglobulin for use against hepatitis A and measles, virus-specific polyclonal human immunoglobulin against cytomegalovirus, hepatitis B, rabies, Respiratory Syncytial Virus (RSV), vaccinia, and varicella-zoster, and the humanized monoclonal antibody Synagis (palivizumab) [7]. Polyclonal immunoglobulin has also been used with various success for diseases caused by other human viruses including parvovirus B19 (PV B19) [10–13], Lassa virus [14,15], West Nile virus [16,17], some enteroviruses [18,19], herpes simplex virus [20], Crimean-Congo haemorrhagic fever virus (CCHFV) [21], Junin virus [22], Severe Acute Respiratory Syndrome-Associated coronavirus (SARS CoV) [23,24] and Human Immunodeficiency Virus (HIV) [25–30].

Although serum polyclonal antibody preparations have been clinically effective in many cases, problems related to toxicity including a risk for allergic reactions, lot to lot variation and uncertain dosing have limited their use [3]. Monoclonal antibodies (mAbs) including chimeric animal-human, humanized and fully human monoclonal antibodies (hmAbs) have lower or absent of immunogenicity, toxicity, and lot to lot variation. Further, the molecular mechanisms of therapeutic efficacy of such antibodies are easier to dissect and they can be engineered to further improve their therapeutic properties. Recently, some mAbs have shown clinical success. The humanized mAb Synagis, which is still the only mAb against a viral disease approved for clinical use by the U.S. Food and Drug Administration (FDA), has been widely used for prevention of RSV infections in neonates and immune-compromised individuals, and very recently has been further improved [31]. However, it is not effective for treatment of an already established infection, e.g. there were no significant differences in clinical outcomes between placebo and palivizumab groups for children hospitalized with RSV infection [32]; in addition, resistance can develop relatively quickly – F gene resistant mutations were found in an animal model of the RSV infection (cotton rat), 12 weeks after infection including a completely resistant virus [33]. Indeed, for enveloped viruses like RSV, it is almost without exception that all neutralizing antibodies are directed against the virus' envelope glycoproteins which project from the surface of the virion particle, and traditionally the antibody response has been the immunologic measure of viral vaccine efficacy.

In the absence of vaccines or effective drugs, the development of hmAbs for prophylaxis and treatment of emerging viruses and viruses of biodefense importance is an important area of therapeutics development. Here we review hmAbs with potential for prophylaxis and treatment of diseases caused by SARS CoV and henipaviruses (Hendra (HeV) and Nipah (NiV)). The primary focus of this review is on immunoglobulin G1 (IgG1). However, other isotypes could be also useful but are not so frequently used and other formats including antigen-binding fragments (Fabs) and single chain variable fragments (scFvs) were sometimes described.

2. Antibodies to SARS CoV, Hendra and Nipah viruses elicited by infection or immunization

The SARS CoV [34–37] caused a world-wide epidemic in 2002 and 2003, and infected more than 8000 humans with a fatality rate of about 10%. Although there are no recent outbreaks, the need to develop potent therapeutics and vaccines against a re-emerging SARS CoV or a related virus remains of high importance.

SARS CoV surface glycoprotein, also called spike glycoprotein, (S protein or S glycoprotein) mediates viral entry into the host cell and has two functional domains S1 and S2. The S1 domain is involved in the binding of the cellular receptor ACE2 whereas the S2 domain facilitates the fusion between viral and host cell membranes. Infections by many viruses, including coronaviruses, elicit potent neutralizing antibodies (nAbs) that can affect the course of infection and help clear the virus; they can also protect an uninfected host exposed to the virus. SARS CoV is no exception - nAbs were detected in SARS CoV-infected patients [38–43], and in mice [44], hamsters [45], and monkeys [46] infected with the virus. These antibodies also protected uninfected animals from SARS CoV infection, e.g., passive transfer of immune serum to naive mice prevented virus replication in the lower respiratory tract following intranasal challenge [44]. NAbs from serum targeted the S protein [43], including epitopes containing portions of the receptor-binding domain (RBD) [47] on S1, conserved fragments from S2, [48], and a limited number of fragments from other regions of S [42]. Their epitopes appear to be both conformational and linear as one study found the association of linear epitopes with S protein C-terminal regions and conformational epitopes with its N-terminal domain [49].

Immunization with various antigens also induces nAb in mice [47,50–57], hamsters [58], rabbits [55,59,60], ferrets [61], pigs [62] and monkeys [63,64]. The S glycoprotein alone can mediate entry of the SARS CoV [43,65] and, therefore, it has been mostly used as an immunogen. It has also been recently found that nAb can be elicited with about equal efficacy by the soluble ectodomain and by the full-length membrane associated S protein using DNA immunization of mice without boosting with protein (Xiao, Biragyn and Dimitrov, unpublished data). In this study, mice were immunized with plasmid DNA encoding full length spike protein, RBD, and a fusion protein of the RBD with Fc. They all elicited high serum titers against the S protein RBD (>10,000 U/ml). Sera from immunized mice also inhibited S protein-mediated cell fusion (> 90% inhibition with 1:20 serum dilution). The neutralizing activities were comparable between sera from animals immunized with different forms of the RBD. These data suggest that the RBD of S protein is a highly immunogenic and contains epitopes of neutralizing antibodies independently of whether it is expressed as a part of the full length protein or soluble RBD. They also confirm the role of the RBD as the immunodominant domain of the S protein and a target for neutralizing antibodies.

Immunization of mice has been used for production of murine neutralizing monoclonal antibodies (nmAbs) [66,67]. Two of the S-specific mAbs (F26G18 and F26G19) demonstrated the highest in vitro neutralizing potency (in the subnanomolar range); it appears that the nmAbs targeted predominantly conformational epitopes [66]. Antibodies from convalescent SARS patients, but not normal human serum, have been also shown to specifically compete off binding of these mAbs to whole SARS CoV indicating the existence of antibodies in infected humans targeting the same or overlapping epitopes as the mouse nmAbs. The most potent murine nmAbs, e.g. F26G18, may have potential for prevention and treatment of SARS especially after humanization of these antibodies to avoid possible immunogenicity effects.

Nipah virus (NiV) and Hendra virus (HeV) are closely related emerging paramyxoviruses that comprise the *Henipavirus* genus [68–77]. The broad species tropisms and the ability to cause

fatal disease in both animals and humans distinguish HeV and NiV from all other known paramyxoviruses (reviewed in [78]). They are Biological Safety Level-4 (BSL-4) pathogens, and are on the NIAID Biodefense research agenda as zoonotic emerging category C priority pathogens that could be used as bioterror agents. There are currently no therapeutic modalities for treating NiV or HeV infections, and a vaccine for prevention of disease in human or livestock populations does not exist. Although antibody responses were detected in infections caused by these viruses, and mouse mAbs have been isolated, the need for development of hmAbs specific for HeV and NiV has only just been realized.

Like SARS CoV, HeV and NiV are also enveloped viruses but they possess two major glycoprotein spikes; an attachment (G) glycoprotein which binds to the virus receptors ephrinB2 and ephrinB3, and a Fusion (F) glycoprotein which facilitates the fusion of virus and host cell membranes (reviewed in [78,79]). Antibodies specific for either the F or G glycoproteins can neutralize virus, but it is those antibodies specific for the G glycoproteins which appear to be the dominant target antigen for neutralizing antibodies. Both animals and humans infected by either HeV or NiV do develop neutralizing antibodies, and very recently, a Bio-Plex Luminex® platform assay was developed in which recombinant soluble G (sG) glycoproteins from both HeV and NiV were used as antigens allowing for the simultaneous detection and differentiation of HeV and NiV specific neutralizing antibodies in sera from naturally infected or immunized sources [80].

Since the F and G glycoproteins are the major target of nAbs, their use as the antigen components of vaccines have been explored. In addition to their natural hosts, flying foxes, NiV has naturally infected pigs, horses, cats, dogs and humans; experimental infections of guinea pigs, hamsters and cats with NiV have also been demonstrated (reviewed in [78]). In contrast, HeV appears less transmissible in nature and naturally acquired infections of only bats, horses and humans have been described, but experimentally HeV will infect guinea pigs and cats (reviewed in [78]). The use of smaller animal models to study pathogenesis and aid in the efficacy studies of vaccines and therapeutics has been problematic for both HeV and NiV. Neither virus appears capable of infecting and causing reproducible disease in mice or rabbits and infection of guinea pigs has also met with variable results, and to date, the only small animal model in use is with NiV in the golden hamster (reviewed in [79]). Presently, among reported findings, the golden hamster and cat represent the available suitable animal models to examine the severe pathogenic processes of NiV or HeV infection and have been used in challenge experiments in the evaluation of potential antiviral therapeutics (reviewed in [79]). A vaccination and challenge study with NiV was carried out in a hamster model using recombinant vaccinia viruses encoding full-length NiV F or G [81]. As expected, this approach did elicit neutralizing antibodies against NiV and was able to protect the animals from lethal disease. Although effective, neither of these platforms is likely to be a viable vaccine candidate for use in humans. Further, because of their BSL4 classification and highly pathogenic nature the development of live-attenuated HeV or NiV vaccines is also doubtful. However, recombinant subunit immunogens could be a viable approach for a NiV or HeV vaccine because they are extremely safe and can be administered with no risk of infection.

Recombinant sG from both HeV and NiV glycoproteins has been explored as a vaccine immunogen. The sG has been shown to retain several structural, functional and antigenic characteristics similar to native G, including oligomerization and receptor binding competence, and can elicit strong polyclonal neutralizing antibody responses in rabbits, mice [79,82]. Cats immunized with either NiV or HeV sG exhibited very high homologous serum neutralizing titers, and although both immunogens elicited significant levels of heterologous (cross-reactive) neutralizing titers, the animals given HeV sG yielded a higher cross-reactive response. All immunized cats were completely protected from a lethal challenge with NiV providing evidence for a successful subunit vaccine but also that a single vaccine (HeV sG) could be used

to protect against both HeV and NiV. Further evidence of the ability of HeV G to elicit a more cross-reactive antibody response was recently provided by Bossart et al., where an analysis of antibody responses in sera from naturally infected or immunized sources has revealed that, although the specificity of the response to G mirrored the infecting virus, HeV-infected individuals had high levels of NiV G cross-reactive antibodies while NiV-infected individuals had limited cross-reactive antibodies to HeV G [80]. Interestingly, as we described in the latter part of text, panning of phage-displayed antibody libraries with HeV G (which mimics some aspects of in vivo immunization) resulted in selection of high-affinity potent neutralizing antibodies against NiV. Together, these characteristics of G and the recombinant sG suggest that it would be an ideal antigen for the purposes of eliciting or isolating neutralizing human monoclonal antibodies (nhmAbs).

In addition to active vaccination, evidence of passively delivered antibody protection against a NiV challenge has been demonstrated in hamsters using pools of monospecific polyclonal antisera against F and G [81]. Although the amounts of serum were large and both sera and virus were administered in close succession by intraperitoneal injection, this study demonstrated that neutralizing antibodies to the viral envelope glycoproteins of NiV can protect against lethal disease. A follow-up study from the same group using two murine mAbs against NiV F and two against G as passive immunotherapies has also been conducted [83]. Using hamsters, the mAbs were administered as ascitic fluid by intraperitoneal injection and several versions of the protocol were carried out, varying the amounts and timing of administration. Animals that received the mAbs in sufficient amounts before and immediately following the intraperitoneal challenge of NiV were completely protected. High levels of either anti-G or anti-F mAbs seemed to afford sterilizing immunity while lower amounts of antibody resulted in measurable increases in anti-NiV antibodies following virus challenge, but could still protect against fatal infection. These studies also support the notion that passive immunotherapy directed against the viral envelope glycoproteins could be a viable treatment for NiV infection. However, a passive immunization experiment using antibody administered systemically before or following virus challenge has yet to be evaluated, and the humanization of these murine mAbs to formulate an acceptable therapeutic product for human use will take considerable effort.

3. Potent neutralizing human monoclonal antibodies against the SARS CoV S glycoprotein

Several groups have recently developed hmAbs to the SARS CoV S glycoprotein that neutralize the virus and have potential for therapy and prophylaxis of SARS (reviewed in [84]). Recently, an improved method for Epstein-Barr Virus (EBV) transformation of human B cells has been developed based on CpG oligonucleotide (CpG 2006) that increases the B cell immortalization efficiency from 1–2% to 30–100%, and this method was used for selection of hmAbs specific for SARS CoV proteins [85]. One of the selected antibodies (S3.1), which was specific for the S glycoprotein on the viral spikes, was about 500-fold more efficient in neutralization than convalescent serum. S3.1 prevented the cytopathic effect of the SARS CoV at 300 ng/ml [85], and inhibited entry of pseudovirus with S glycoprotein from the Urbani isolate with about the same IC_{50} . However, it did not affect to any significant extent pseudovirus entry mediated by the GD03T0013 isolate S glycoprotein and even enhanced the entry of virus pseudotyped with the S glycoprotein from the palm civet isolate SZ16 [86]. In a mouse model of SARS CoV infection this antibody prevented viral replication in the lower respiratory tract (at doses of 200 μ g and 800 μ g), and reduced it in the upper respiratory tract at the highest dose (800 μ g) used. But, data for the in vivo neutralizing activity of other nhmAbs selected in this study [85] including the most potent antibody (S215.13), which has a neutralizing concentration (1 ng/ml) 300-fold lower than that of S3.1, have not been reported

in this article. The high neutralizing activities of these two hmAbs in IgG1 format indicates possibilities for their use alone or in combination for prophylaxis and treatment of SARS.

Phage display technology has been increasingly used to produce high affinity hmAbs from both naïve and immune libraries. An advantage of using a naïve library is that B lymphocytes from an infected or immunized host are not required. Recently, two human nonimmune scFv libraries containing about 10^{10} members were developed from B cells of unimmunized donors, and used for selection of antibodies against a purified S fragment containing residues 12–672 [87]. One of the selected antibodies, IgG1 80R, can neutralize 50% of the virus in a microneutralization assay at a concentration as low as 0.37 nM. It also blocked formation of syncytia, which could contribute to the spread of the virus in vivo, although at significantly higher concentration (25 nM). Its epitope overlaps the binding site of the SARS CoV receptor ACE2 suggesting a possible mechanism of neutralization by preventing the virus attachment to its receptor [87,88]. When 80R IgG1 was given prophylactically to mice at doses therapeutically achievable in humans, viral replication was reduced to below assay limits [89]. One should note that the conditions used for evaluation of the neutralizing activity of different antibodies in this study are not exactly the same as in the study described above and also below; thus comparing the activity of different antibodies should be done with caution unless they are tested side by side under exactly the same conditions.

Three neutralizing hmAbs were also selected from another large naïve antibody library [90, 91]. They bound a recombinant S1 fragment comprising amino acid residues 318 to 510, a region previously identified as the SARS RBD (SRBD) [91]. The most potent of these hnmAb, IgG1 CR3014, required the residue Asn479 of S RBD for its binding [91]. This antibody exhibited in vitro 50% neutralizing activity at about 1 µg/ml. More importantly, this antibody showed neutralizing activity in ferrets. In one set of experiments ferrets were inoculated either with virus at two doses (low – 10^3 TCID₅₀ and high - 10^4 TCID₅₀) or with virus preincubated with the antibody at 0.13 mg/ml for the low dose and 1.3 mg/ml for the high one. Animals exposed to the virus-antibody mixture had almost undetectable SARS CoV in the lung, showed no lung lesions on day 4 or 7, and did not shed virus in their throats unlike control animals treated with irrelevant antibody. In a second set of experiments the antibody at 10 mg/kg was administered 24 h before challenge with virus and reached 65–84 µg/ml serum concentration in three of the animals (< 5 µg/ml in the fourth one). In the three ferrets with high antibody concentration virus shedding in the throat was completely abolished while in the fourth one it was comparable to that of the control group. The CR3014 treated animals had 3.3 logs lower mean virus titer than the controls, and were completely protected from macroscopic lung pathology. Note that the antibody dose used (10 mg/kg) was less than the one (15 mg/kg) used for prevention of RSV infections in infants which is administered once a month. Recently, in vitro SARS-CoV variants escaping neutralization by CR3014 were generated that all had a single Pro462Leu mutation in the S glycoprotein of the escape virus [92]. In vitro experiments confirmed that binding of CR3014 to a recombinant S fragment (amino acid residues 318–510) harboring this mutation was abolished. An antibody-phage library derived from blood of a convalescent SARS patient was screened for antibodies complementary to CR3014. A novel mAb, CR3022, was identified that neutralized CR3014 escape viruses, did not compete with CR3014 for binding to recombinant S1 fragments, and bound to S1 fragments derived from the civet cat SARS-CoV-like strain SZ3. No escape variants could be generated with CR3022. The mixture of both mAbs showed neutralization of SARS-CoV in a synergistic fashion by recognizing different epitopes on the RBD but the mechanism remains unknown. Dose reduction indices of 4.5 and 20.5 were observed for CR3014 and CR3022, respectively, at 100% neutralization [92]. These results suggest a potential use of CR3014 in combination with CR3022 for prophylaxis of SARS CoV infections in humans. However, one should note that currently there is no available animal model of the SARS CoV infection that results in death as in humans.

Two other hmAbs (201 and 68) were derived from transgenic mice with human Ig genes and evaluated in a murine model of SARS CoV infection [93]. One of these antibodies 201 bound within the RBD of the S protein at amino acid residues 490–510 while the other one 68 bound to a region containing residues 130–150. In a microneutralization assay based on protection to cytopathic effects the IC₅₀ for 201 was about 0.2 µg/ml [93]. Mice that received 40 mg/kg of these antibodies prior to challenge with the SARS CoV were completely protected from virus replication in the lungs, and doses as low as 1.6 mg/kg offered significant protection. These antibodies have potential as therapeutics and research tools [93].

Recently, a novel cross-reactive potent SARS CoV-neutralizing hmAb, m396, was identified by using a fragment containing residues 317 through 518 as a selecting antigen for panning of a large human antibody library constructed from the B lymphocytes of healthy volunteers [94]; this fragment was previously identified to contain the RBD [95–97], which is a major SARS CoV neutralization determinant [84,98–103]. Because the SARS CoV caused two outbreaks - the epidemic in late 2002/early 2003 and a second outbreak in the winter of 2003–2004 by an independent animal-to-human transmission – the antibody m396 was tested against isolates from both outbreaks and from animal hosts that initiated these outbreaks. The GD03 strain, which was isolated from an index patient of the second outbreak, was reported to resist neutralization by the hmAbs 80R and S3.1 which can potentially neutralize isolates from the first outbreak. It was found that m396 and another antibody, S230.15, potentially neutralized GD03 and representative isolates from the first SARS outbreak (Urbani, Tor2) and from palm civets (SZ3, SZ16) [104]. These antibodies also protected mice challenged with the Urbani, or recombinant viruses bearing the GD03 and SZ16 S glycoproteins. Both antibodies competed with the SARS CoV receptor, ACE2, for binding to the RBD suggesting a mechanism of neutralization that involves interference with the SARS CoV-ACE2 interaction. These antibodies are the first identified hmAbs, which exhibit cross-reactivity against isolates from the two SARS outbreaks and palm civets, and could have potential applications for diagnosis, prophylaxis and treatment of SARS CoV infections.

NABs directed to S inhibit SARS CoV entry either by interfering typically with the S RBD-receptor interactions [87] or by other mechanisms including binding to other portions of S. A human scFv, B1, was identified, which recognizes an epitope on S2 protein located within amino acids 1023–1189 [105]. This antibody recognized SARS pseudovirus in vivo and competed with SARS sera for binding to SARS CoV with an equilibrium dissociation constant, K_d = 105 nM. The B1 also had potent neutralizing activities against infection by pseudovirus expressing SARS CoV S protein in vitro. Other mechanisms of SARS CoV infection inhibition could include steric hindrance that indirectly prevents virus attachment to receptors and binding to entry intermediates. Mechanisms that could operate in vivo, and for lack of data will not be discussed here, are related to the antibody biological effector functions conferred by the antibody Fc, e.g., antibody-dependent cellular cytotoxicity (ADCC).

The S-ACE2 interactions can be blocked by antibodies targeting either S or ACE2. Indeed, it was found that antibodies to ACE2 but not an anti-ACE1 antibody blocked viral replication on Vero E6 cells [106]. Because the receptor is a host molecule, which does not mutate, the use of antibodies targeting receptor molecules may prevent the generation of resistant mutants. However, it appears that for SARS CoV infection, which is an acute infection, generation of resistant mutants may not be a significant problem although the recent identification of bats as a natural reservoir of SARS CoV suggests the possibility for large variations in sequences of viruses that could be transmitted to humans [107,108]. In addition, such anti-ACE2 antibodies could affect the function of ACE2-expressing cells. Experiments in animal models are required to find whether SARS CoV infection in presence of nhmAbs will lead to generation of neutralization escape mutants and whether anti-ACE2 nhmAbs antibodies have deleterious effects on the host.

4. Molecular mechanisms of SARS CoV neutralization and cross-reactivity

In recent years, a substantial amount of structural information on the mechanisms of SARS CoV entry, neutralization and cross-reactivity has been made available. The crystal structure of the S RBD-ACE2 complex [109] revealed the structure of RBD and its interactions with the receptor (Figure 1A). The RBD has a five-stranded anti-parallel β -sheet core ($\beta 1$ – $\beta 4$ and $\beta 7$), with an extended loop supported by a two-stranded β -sheet ($\beta 5$ and $\beta 6$) segregated separately from the core. The S RBD-ACE2 receptor interactions bury about 1700 Å² at the interface and are predominantly made by hydrophilic side chains including six tyrosines at the RBD. The crystal structure of Fab m396 in complex with the S RBD was determined at 2.3-Å resolution (Figure 1B) [94]. The m396 antibody epitope is dominated by a 10-residue-long protruding $\beta 6$ – $\beta 7$ loop. The $\beta 6$ – $\beta 7$ loop comprising residues 482–491 that prominently protrude from the RBD surface contacts four of the CDRs (complementarity-determining-regions) of Fab m396, H1, H2, H3, and L3. A total surface area of 1760 Å² is buried at the interface of the complex and the antibody-binding $\beta 6$ – $\beta 7$ loop alone accounts for 63% of the RBD-antibody interface. The crystal structure of anti-SARS scFv 80R antibody in complex with the S RBD was also determined [88]. In the 80R antibody complex structure (Figure 1C), the S RBD-80R interface buries a relatively large surface of 2200 Å² and the binding of 80R involves all the six CDRs loops as well as the major portion of framework region between the L2 and L3 loops. Comparison between these RBD-ACE2, RBD-m396 and RBD-80R complex structures provides important clues for the structural mechanism of antibody-mediated neutralization and cross-reactivity. It is observed that antibodies Fab 396 and scFv 80R as well as the ACE2 receptor occupy a common region on the surface of RBD (colored red in Figure 1A–C), which mainly consists of the $\beta 6$ – $\beta 7$ loop of RBD. Therefore, most of the residues in that loop of RBD are critical for the binding to both the antibody and the receptor. Close-up views of the $\beta 6$ – $\beta 7$ loop of RBD in the corresponding S RBD complexes are shown in Figure 1D–F where the Tyr491 residue in the loop plays a critical role in contacting the receptor and antibodies. In the ACE2 receptor complex, Tyr491 of RBD is in contact with Lys353 (Figure 1D) that determines the species specificity of ACE2; Lys353 for human and civet where as His353 for mouse and rat. In the antibody complexes, Tyr491 of RBD binds into the antibody combining sites through CDR loops of their heavy chains (Figure 1E–F). These observations demonstrate that the antibody neutralizes SARS CoV by competing for the same set of critical residues in the $\beta 6$ – $\beta 7$ loop of RBD and therefore blocking the receptor binding site. It is noted that the residues Tyr491 and Ile489 were identified as putative hot spots in the RBD within the SARS CoV spike that likely contribute to most of the m396 binding energy. These residues are highly conserved within the SARS CoV spike indicating a possible mechanism of the m396 cross-reactivity. Further, sequence analysis and mutagenesis data showed that m396 might neutralize all zoonotic and epidemic SARS CoV isolates with known sequences, except strains derived from bats.

Intriguingly, either the unliganded RBD [88] (not depicted here) or the antibody-bound RBD [88,93] are not significantly different from that of the ACE2-bound RBD [109] which suggests a lock-and-key mechanism at the antibody binding site, and lack of RBD conformational changes induced by the S1 domain of ACE2 itself with important implications for the mechanism of entry. Further, the major neutralizing determinants are located contiguously in one major segment of the $\beta 6$ – $\beta 7$ loop in antibody m396 complex; whereas the other antibody 80R and receptor ACE2 have determinants over most of the extended loop appearing on the top of RBD. This difference leads to a critical effect on the differential specificity and sensitivity of these antibodies. For example, Asp480 of S RBD is found to be a contact residue in 80R that Asp480Ala or Asp480Gly mutation completely abrogates the binding to 80R. This mutation does not affect the m396 antibody binding as the epitope does not include this residue.

Antibody m396 potently neutralizes virus pseudotyped with the S glycoprotein from the 2003/2004 Guangdong index patient (GD03T0013 isolate). Compared to the middle/late phase 2002/2003 isolate Tor2, five residues in the RBD were mutated in the late isolate GD03T0013, including Lys344Arg, Phe360Ser, Leu472Pro, Asp480Gly, and Thr487Ser. Among the five residues, Thr487 only contacts the antibody combining site as observed in the RBD-m396 structure. However, in the RBD-m396 complex structure, Thr487 makes only a backbone contact and therefore the Thr487Ser mutation would not affect significantly its binding to the antibody. Thus, being able to neutralize all SARS CoV isolates with known sequences, m396 appears to be a broadly cross-reactive neutralizing antibody against SARS CoV [104].

5. Potent neutralizing human monoclonal antibodies against the Nipah virus (NiV) and Hendra virus (HeV)

The identification of potent neutralizing hmAbs targeting the viral envelope glycoprotein G by using a highly purified, oligomeric, soluble HeV G (sG) glycoprotein as the antigen for screening of a large naïve human phage-display library was recently reported [110]. The selected seven Fabs, m101-7, inhibited, to various degrees, cell fusion mediated by the HeV or NiV Envs and virus infection. The conversion of the most potent neutralizer of infectious HeV, Fab m101, to IgG1 significantly increased its cell fusion inhibitory activity - the IC₅₀ was decreased more than 10-fold to approximately 1 µg/ml. The IgG1 m101 was also exceptionally potent in neutralizing infectious HeV; complete (100 %) neutralization was achieved with 12.5 µg/ml and 98 % neutralization required only 1.6 µg/ml. The inhibition of fusion and infection correlated with binding of the Fabs to full-length G as measured by immunoprecipitation, and less with binding to sG as measured by ELISA and Biacore. Antibodies m101 and m102 competed with the ephrin-B2, which was identified as a functional receptor for both HeV and NiV [111,112], indicating a possible mechanism of neutralization by these antibodies. It was observed that m101, m102 and m103 antibodies competed with each other suggesting that they bind to overlapping epitopes which are distinct from the epitopes of m106 and m107.

In an initial attempt to localize the epitopes of m101 and m102, we have analyzed the solved complex structures of Nipah and Hendra attachment G glycoproteins with their receptors. Crystal structures of NiV G – ephrin-B3 complex [113] and HeV G – ephrin-B2 complex [114] are depicted in Figure 2A and 2B respectively. NiV-G has a hydrophobic pocket for Tyr120 of ephrin-B3 that was formed by residues Ala558, Gln559, Ile580, Tyr581, and Ile588. Tyr120 residue from the G–H loop of ephrin-B3 fits into the hydrophobic pocket on the surface of NiV G glycoprotein (Figure 2A). HeV-G has also a similar hydrophobic pocket where Phe120 from the G–H loop of ephrin-B2 inserts (Figure 2B). We noted some similarities in these virus-receptor structural interactions to that of HIV gp120 glycoprotein with its receptor CD4 [115] as well as HIV gp120 interactions with the b12 antibody [116]. In the HIV gp120-CD4 interactions, Phe43 of C'C" loop from the CD4 receptor makes a critical contact to gp120 and reaches the hydrophobic pocket formed in between the inner and outer domains of gp120 (Figure 2C). In the gp120 complex with b12 antibody, Tyr53 from the CDR H2 loop of b12 antibody fits into the hydrophobic pocket on the gp120 surface in a similar manner as “Phe43” of CD4 receptor interacts with the gp120 (Figure 2D). Comparison of these glycoprotein complex structures with receptor and antibody would suggest that the epitopes of m101 and m102 might overlap with the binding sites of Tyr120/Phe120 in NiV/HeV G attachment glycoproteins or those with close proximities to these residues that could affect sterically blocking the ephrin receptor binding.

Recently, an affinity matured version of the cross reactive antibody m102 (designated as m102.4) was developed through chain shuffling and phage display library panning [117]. The antibody m102.4 showed significantly improved neutralizing activity against both Nipah and

Hendra viruses in vitro with IC50s below 0.04 and 0.6 µg/ml, respectively. These results suggest that m101, m106 and especially m102.4 exhibit potent cross-reactive neutralizing activities against both Nipah virus and Hendra virus; they are the first hmAbs identified against these viruses and could be used for treatment, prophylaxis, and diagnosis, and as research reagents and aid in the development of vaccines.

6. Conclusions and expert opinion

The hmAbs directed to the SARS CoV S glycoprotein and the henipavirus G glycoprotein are currently in the most advanced stage of development and offer the best hope as potential therapeutics. These antibodies specific for SARS CoV, HeV and NiV have potential for further development into clinically useful products for prophylaxis and perhaps treatment of the diseases caused by these infections. They are very potent, cross-reactive and the viral infections to which they are specific are acute, such that only control or dampening of virus replication for relatively short period of time (week or two) is likely to be required, after which the host immune system could control virus replication. Thus, the problem of neutralization resistant mutants able to evade their inhibitory activity and the immune response is not as significant as for chronic infections with high level of virus replication, e.g. HIV infection. A note of caution is that careful examination of candidate antibody therapeutics is required because of the possibility for infection enhancing effects and animal model-dependent effects as well as in some cases, although rare, toxicity; a recent study also reported the possibility that neutralizing antibodies can enhance entry of SARS CoV by a mechanism that involves antibody interactions with conformational epitopes in the S RBD [86]. In addition, it is known that in some cases antibodies that do not neutralize in the assay currently used for evaluation of their in vitro activity could exhibit potent neutralizing activity in vivo; thus new approaches should be developed and antibodies to be tested also for their effector functions mediated by the Fc including ADCC and complement mediated immune responses. However, only further exploration of these antibodies and their extensive evaluation in animal models likely in combination with other antiviral drugs (antibodies or small molecules) would allow identification of the best candidates for potential therapeutics.

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Reference List

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Dimitrov DS. Virus entry: molecular mechanisms and biomedical applications. *Nat Rev Microbiol* 2004;2:109–122.122 [PubMed: 15043007] Review on virus entry focusing on molecular mechanisms and applications to vaccines and inhibitors, including antibodies.
2. Casadevall A, Dadachova E, Pirofski LA. Passive antibody therapy for infectious diseases. *Nat Rev Microbiol* 2004;2:695–703.703 [PubMed: 15372080] Excellent review on antibody therapy.
3. Casadevall A. Passive antibody therapies: progress and continuing challenges. *Clin Immunol* 1999;93:5–15. [PubMed: 10497006]
4. Casadevall A, Scharff MD. Return to the past: the case for antibody-based therapies in infectious diseases. *Clin Infect Dis* 1995;21:150–161. [PubMed: 7578724]

5. Casadevall A, Scharff MD. Serum therapy revisited: animal models of infection and development of passive antibody therapy. *Antimicrob Agents Chemother* 1994;38:1695–1702. [PubMed: 7985997]
6. Zeitlin L, Cone RA, Moench TR, Whaley KJ. Preventing infectious disease with passive immunization. *Microbes Infect* 2000;2:701–708. [PubMed: 10884621]
7. Sawyer LA. Antibodies for the prevention and treatment of viral diseases. *Antiviral Res* 2000;47:57–77.77 [PubMed: 10996394] Excellent review on antibodies for prevention and treatment of viral diseases.
8. Bayry J, Lacroix-Desmazes S, Kazatchkine MD, Kaveri SV. Intravenous immunoglobulin for infectious diseases: back to the pre-antibiotic and passive prophylaxis era? *Trends Pharmacol Sci* 2004;25:306–310. [PubMed: 15165745]
9. DesJardin JA, Snyderman DR. Antiviral immunotherapy - a review of current status. *Biodrugs* 1998;9:487–507. [PubMed: 18020581]
10. Moudgil A, Shidban H, Nast CC, et al. Parvovirus B19 infection-related complications in renal transplant recipients: treatment with intravenous immunoglobulin. *Transplantation* 1997;64:1847–1850. [PubMed: 9422430]
11. Kurtzman G, Frickhofen N, Kimball J, et al. Pure red-cell aplasia of 10 years' duration due to persistent parvovirus B19 infection and its cure with immunoglobulin therapy. *N Engl J Med* 1989;321:519–523. [PubMed: 2548098]
12. Kerr JR, Cunniffe VS, Kelleher P, et al. Successful intravenous immunoglobulin therapy in 3 cases of parvovirus B19-associated chronic fatigue syndrome. *Clin Infect Dis* 2003;36:e100–e106. [PubMed: 12715326]
13. Koduri PR, Kumapley R, Valladares J, Teter C. Chronic pure red cell aplasia caused by parvovirus B19 in AIDS: use of intravenous immunoglobulin--a report of eight patients. *Am J Hematol* 1999;61:16–20. [PubMed: 10331506]
14. Clayton AJ. Lassa immune serum. *Bull World Health Organ* 1977;55:435–439. [PubMed: 304386]
15. Krasnianskii VP, Gradoboev VN, Borisevich IV, et al. Development and study of properties of immunoglobulins against Lassa fever. *Vopr Virusol* 1997;42:168–171. [PubMed: 9304297]
16. Shimoni Z, Niven MJ, Pitlick S, Bulvik S. Treatment of West Nile virus encephalitis with intravenous immunoglobulin. *Emerg Infect Dis* 2001;7:759. [PubMed: 11585547]
17. Hamdan A, Green P, Mendelson E, et al. Possible benefit of intravenous immunoglobulin therapy in a lung transplant recipient with West Nile virus encephalitis. *Transpl Infect Dis* 2002;4:160–162. [PubMed: 12421462]
18. Pasic S, Jankovic B, Abinun M, Kanjuh B. Intravenous immunoglobulin prophylaxis in an echovirus 6 and echovirus 4 nursery outbreak. *Pediatr Infect Dis J* 1997;16:718–720. [PubMed: 9239781]
19. Rotbart HA, O'Connell JF, McKinlay MA. Treatment of human enterovirus infections. *Antiviral Res* 1998;38:1–14. [PubMed: 9613999]
20. Masci S, De Simone C, Famularo G, et al. Intravenous immunoglobulins suppress the recurrences of genital herpes simplex virus: a clinical and immunological study. *Immunopharmacol Immunotoxicol* 1995;17:33–47. [PubMed: 7759773]
21. Vassilenko SM, Vassilev TL, Bozadjiev LG, et al. Specific intravenous immunoglobulin for Crimean-Congo haemorrhagic fever. *Lancet* 1990;335:791–792. [PubMed: 1969533]
22. Enria DA, Briggiler AM, Fernandez NJ, et al. Importance of dose of neutralising antibodies in treatment of Argentine haemorrhagic fever with immune plasma. *Lancet* 1984;2:255–256. [PubMed: 6146809]
23. Ali MB. Treating severe acute respiratory syndrome with hyperimmune globulins. *Hong Kong Med J* 2003;9:391–392. [PubMed: 14530539]
24. Burnouf T, Radosevich M. Treatment of severe acute respiratory syndrome with convalescent plasma. *Hong Kong Med J* 2003;9:309. [PubMed: 12904626]
25. Vittecoq D, Chevret S, Morand-Joubert L, et al. Passive immunotherapy in AIDS: a double-blind randomized study based on transfusions of plasma rich in anti-human immunodeficiency virus 1 antibodies vs. transfusions of seronegative plasma. *Proc Natl Acad Sci U S A* 1995;92:1195–1199. [PubMed: 7862660]
26. Jablonowski H, Sander O, Willers R, et al. The use of intravenous immunoglobulins in symptomatic HIV infection. Results of a randomized study. *Clin Investig* 1994;72:220–224.

27. Olopoenia L, Young M, White D, et al. Intravenous immunoglobulin in symptomatic and asymptomatic children with perinatal HIV infection. *J Natl Med Assoc* 1997;89:543–547. [PubMed: 9264222]
28. Guay LA, Musoke P, Hom DL, et al. Phase I/II trial of HIV-1 hyperimmune globulin for the prevention of HIV-1 vertical transmission in Uganda. *AIDS* 2002;16:1391–1400. [PubMed: 12131216]
29. Dezube BJ, Proper J, Zhang J, et al. A passive immunotherapy, (PE)HRG214, in patients infected with human immunodeficiency virus: a phase I study. *J Infect Dis* 2003;187:500–503. [PubMed: 12552435]
30. Zolla-Pazner S, Gorny MK. Passive immunization for the prevention and treatment of HIV infection. *AIDS* 1992;6:1235–1247. [PubMed: 1472330]
31. Wu H, Pfarr DS, Tang Y, et al. Ultra-potent antibodies against respiratory syncytial virus: effects of binding kinetics and binding valence on viral neutralization. *J Mol Biol* 2005;350:126–144. [PubMed: 15907931] Perhaps the most potent antibody of potential clinical use against respiratory syncytial virus.
32. Saez-Llorens X, Moreno MT, Ramilo O, et al. Safety and pharmacokinetics of palivizumab therapy in children hospitalized with respiratory syncytial virus infection. *Pediatr Infect Dis J* 2004;23:707–712. [PubMed: 15295219]
33. Zhao X, Sullender WM. In vivo selection of respiratory syncytial viruses resistant to palivizumab. *J Virol* 2005;79:3962–3968. [PubMed: 15767398]
34. Ksiazek TG, Erdman D, Goldsmith CS, et al. A novel coronavirus associated with severe acute respiratory syndrome. *N Engl J Med* 2003;348:1953–1966. [PubMed: 12690092] First identification of SARS coronavirus.
35. Peiris JS, Lai ST, Poon LL, et al. Coronavirus as a possible cause of severe acute respiratory syndrome. *Lancet* 2003;361:1319–1325. [PubMed: 12711465]
36. Drosten C, Gunther S, Preiser W, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *N Engl J Med* 2003;348:1967–1976. [PubMed: 12690091] First identification of SARS coronavirus.
37. Holmes KV. SARS-associated coronavirus. *N Engl J Med* 2003;348:1948–1951. [PubMed: 12748314]
38. Hsueh PR, Huang LM, Chen PJ, et al. Chronological evolution of IgM, IgA, IgG and neutralisation antibodies after infection with SARS-associated coronavirus. *Clin Microbiol Infect* 2004;10:1062–1066. [PubMed: 15606632]
39. Nie Y, Wang G, Shi X, et al. Neutralizing antibodies in patients with severe acute respiratory syndrome-associated coronavirus infection. *J Infect Dis* 2004;190:1119–1126. [PubMed: 15319862]
40. Shi Y, Wan Z, Li L, et al. Antibody responses against SARS-coronavirus and its nucleocapsid in SARS patients. *J Clin Virol* 2004;31:66–68. [PubMed: 15288616]
41. Han DP, Kim HG, Kim YB, et al. Development of a safe neutralization assay for SARS-CoV and characterization of S-glycoprotein. *Virology* 2004;326:140–149. [PubMed: 15262502]
42. Guo JP, Petric M, Campbell W, McGeer PL. SARS corona virus peptides recognized by antibodies in the sera of convalescent cases. *Virology* 2004;324:251–256. [PubMed: 15207612]
43. Hofmann H, Hattermann K, Marzi A, et al. S protein of severe acute respiratory syndrome-associated coronavirus mediates entry into hepatoma cell lines and is targeted by neutralizing antibodies in infected patients. *J Virol* 2004;78:6134–6142. [PubMed: 15163706]
44. Subbarao K, McAuliffe J, Vogel L, et al. Prior infection and passive transfer of neutralizing antibody prevent replication of severe acute respiratory syndrome coronavirus in the respiratory tract of mice. *J Virol* 2004;78:3572–3577. [PubMed: 15016880]
45. Roberts A, Vogel L, Guarner J, et al. Severe acute respiratory syndrome coronavirus infection of golden Syrian hamsters. *J Virol* 2005;79:503–511. [PubMed: 15596843]
46. McAuliffe J, Vogel L, Roberts A, et al. Replication of SARS coronavirus administered into the respiratory tract of African Green, rhesus and cynomolgus monkeys. *Virology* 2004;330:8–15. [PubMed: 15527829]
47. Zhou T, Wang H, Luo D, et al. An exposed domain in the severe acute respiratory syndrome coronavirus spike protein induces neutralizing antibodies. *J Virol* 2004;78:7217–7226. [PubMed: 15194798]

48. Zhang H, Wang G, Li J, et al. Identification of an antigenic determinant on the S2 domain of the severe acute respiratory syndrome coronavirus spike glycoprotein capable of inducing neutralizing antibodies. *J Virol* 2004;78:6938–6945. [PubMed: 15194770]
49. Yao YX, Ren J, Heinen P, et al. Cleavage and serum reactivity of the severe acute respiratory syndrome coronavirus spike protein. *J Infect Dis* 2004;190:91–98. [PubMed: 15195247]
50. Zeng F, Chow KY, Hon CC, et al. Characterization of humoral responses in mice immunized with plasmid DNAs encoding SARS-CoV spike gene fragments. *Biochem Biophys Res Commun* 2004;315:1134–1139. [PubMed: 14985131]
51. Yang ZY, Kong WP, Huang Y, et al. A DNA vaccine induces SARS coronavirus neutralization and protective immunity in mice. *Nature* 2004;428:561–564. [PubMed: 15024391]
52. Bisht H, Roberts A, Vogel L, et al. Severe acute respiratory syndrome coronavirus spike protein expressed by attenuated vaccinia virus protectively immunizes mice. *Proc Natl Acad Sci U S A* 2004;101:6641–6646. [PubMed: 15096611]
53. Qu D, Zheng B, Yao X, et al. Intranasal immunization with inactivated SARS-CoV (SARS-associated coronavirus) induced local and serum antibodies in mice. *Vaccine* 2005;23:924–931. [PubMed: 15603894]
54. Xiong S, Wang YF, Zhang MY, et al. Immunogenicity of SARS inactivated vaccine in BALB/c mice. *Immunol Lett* 2004;95:139–143. [PubMed: 15388253]
55. He Y, Zhou Y, Wu H, et al. Identification of immunodominant sites on the spike protein of severe acute respiratory syndrome (SARS) coronavirus: implication for developing SARS diagnostics and vaccines. *J Immunol* 2004;173:4050–4057. [PubMed: 15356154]
56. Takasuka N, Fujii H, Takahashi Y, et al. A subcutaneously injected UV-inactivated SARS coronavirus vaccine elicits systemic humoral immunity in mice. *Int Immunol* 2004;16:1423–1430. [PubMed: 15314040]
57. Tang L, Zhu Q, Qin E, et al. Inactivated SARS-CoV vaccine prepared from whole virus induces a high level of neutralizing antibodies in BALB/c mice. *DNA Cell Biol* 2004;23:391–394. [PubMed: 15231072]
58. Buchholz UJ, Bukreyev A, Yang L, et al. Contributions of the structural proteins of severe acute respiratory syndrome coronavirus to protective immunity. *Proc Natl Acad Sci U S A* 2004;101:9804–9809. [PubMed: 15210961]
59. He Y, Zhou Y, Siddiqui P, Jiang S. Inactivated SARS-CoV vaccine elicits high titers of spike protein-specific antibodies that block receptor binding and virus entry. *Biochem Biophys Res Commun* 2004;325:445–452. [PubMed: 15530413]
60. Pang H, Liu Y, Han X, et al. Protective humoral responses to severe acute respiratory syndrome-associated coronavirus: implications for the design of an effective protein-based vaccine. *J Gen Virol* 2004;85:3109–3113. [PubMed: 15448374]
61. Weingartl H, Czub M, Czub S, et al. Immunization with modified vaccinia virus Ankara-based recombinant vaccine against severe acute respiratory syndrome is associated with enhanced hepatitis in ferrets. *J Virol* 2004;78:12672–12676. [PubMed: 15507655]
62. Weingartl HM, Copps J, Drebot MA, et al. Susceptibility of pigs and chickens to SARS coronavirus. *Emerg Infect Dis* 2004;10:179–184. [PubMed: 15030680]
63. Gao W, Tamin A, Soloff A, et al. Effects of a SARS-associated coronavirus vaccine in monkeys. *Lancet* 2003;362:1895–1896. [PubMed: 14667748]
64. Bukreyev A, Lamirande EW, Buchholz UJ, et al. Mucosal immunisation of African green monkeys (*Cercopithecus aethiops*) with an attenuated parainfluenza virus expressing the SARS coronavirus spike protein for the prevention of SARS. *Lancet* 2004;363:2122–2127. [PubMed: 15220033]
65. Xiao X, Dimitrov DS. The SARS-CoV S glycoprotein. *Cell Mol Life Sci* 2004;61:2428–2430. [PubMed: 15526150]
66. Berry JD, Jones S, Drebot MA, et al. Development and characterisation of neutralising monoclonal antibody to the SARS-coronavirus. *J Virol Methods* 2004;120:87–96. [PubMed: 15234813]
67. Gubbins MJ, Plummer FA, Yuan XY, et al. Molecular characterization of a panel of murine monoclonal antibodies specific for the SARS-coronavirus. *Mol Immunol* 2005;42:125–136. [PubMed: 15488951]

68. Murray K, Selleck P, Hooper P, et al. A morbillivirus that caused fatal disease in horses and humans. *Science* 1995;268:94–97. [PubMed: 7701348]
69. Lee KE, Umapathi T, Tan CB, et al. The neurological manifestations of Nipah virus encephalitis, a novel paramyxovirus. *Ann Neurol* 1999;46:428–432. [PubMed: 10482278]
70. Lim CC, Sitoh YY, Hui F, et al. Nipah viral encephalitis or Japanese encephalitis? MR findings in a new zoonotic disease. *AJNR Am J Neuroradiol* 2000;21:455–461. [PubMed: 10730635]
71. Chua KB, Goh KJ, Wong KT, et al. Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. *Lancet* 1999;354:1257–1259. [PubMed: 10520635]
72. Chew MH, Arguin PM, Shay DK, et al. Risk factors for Nipah virus infection among abattoir workers in Singapore. *J Infect Dis* 2000;181:1760–1763. [PubMed: 10823780]
73. Chua KB, Lam SK, Tan CT, et al. High mortality in Nipah encephalitis is associated with presence of virus in cerebrospinal fluid. *Ann Neurol* 2000;48:802–805. [PubMed: 11079547]
74. Goh KJ, Tan CT, Chew NK, et al. Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. *N Engl J Med* 2000;342:1229–1235. [PubMed: 10781618]
75. Update: outbreak of Nipah virus--Malaysia and Singapore, 1999. *MMWR Morb Mortal Wkly Rep* 1999;48:335–337. [PubMed: 10366143]
76. Chua KB, Bellini WJ, Rota PA, et al. Nipah virus: a recently emergent deadly paramyxovirus. *Science* 2000;288:1432–1435. [PubMed: 10827955]
77. Eaton BT. Introduction to Current focus on Hendra and Nipah viruses. *Microbes Infect* 2001;3:277–278. [PubMed: 11334744]
78. Eaton BT, Broder CC, Middleton D, Wang LF. Hendra and Nipah viruses: different and dangerous. *Nat Rev Microbiol* 2006;4:23–35. [PubMed: 16357858]
79. Bossart KN, Broder CC. Developments towards effective treatments for Nipah and Hendra virus infection. *Expert Rev Anti Infect Ther* 2006;4:43–55. [PubMed: 16441208]
80. Bossart KN, McEachern JA, Hickey AC, et al. Neutralization assays for differential henipavirus serology using Bio-Plex protein array systems. *J Virol Methods* 2007;142:29–40. [PubMed: 17292974]
81. Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. *J Virol* 2004;78:834–840. [PubMed: 14694115]
82. Bossart KN, Crameri G, Dimitrov AS, et al. Receptor binding, fusion inhibition, and induction of cross-reactive neutralizing antibodies by a soluble G glycoprotein of Hendra virus. *J Virol* 2005;79:6690–6702. [PubMed: 15890907]
83. Guillaume V, Contamin H, Loth P, et al. Antibody prophylaxis and therapy against Nipah virus infection in hamsters. *J Virol* 2006;80:1972–1978. [PubMed: 16439553]
84. Zhang MY, Choudhry V, Xiao X, Dimitrov DS. Human monoclonal antibodies to the S glycoprotein and related proteins as potential therapeutics for SARS. *Curr Opin Mol Ther* 2005;7:151–156. [PubMed: 15844623]
85. Traggiai E, Becker S, Subbarao K, et al. An efficient method to make human monoclonal antibodies from memory B cells: potent neutralization of SARS coronavirus. *Nat Med* 2004;10:871–875.875 [PubMed: 15247913] A new efficient method for identification of human monoclonal antibodies directly using B cells from human and development of very potent neutralizers of severe acute respiratory syndrome coronavirus (SCV).
86. Yang ZY, Werner HC, Kong WP, et al. Evasion of antibody neutralization in emerging severe acute respiratory syndrome coronaviruses. *Proc Natl Acad Sci U S A* 2005;102:797–801.801 [PubMed: 15642942] Report of virus entry enhancement of SCV S RBD specific antibody in vitro.
87. Sui J, Li W, Murakami A, et al. Potent neutralization of severe acute respiratory syndrome (SARS) coronavirus by a human mAb to S1 protein that blocks receptor association. *Proc Natl Acad Sci U S A* 2004;101:2536–2541. [PubMed: 14983044]
88. Hwang WC, Lin YQ, Santelli E, et al. Structural basis of neutralization by a human anti-severe acute respiratory syndrome spike protein antibody, 80R. *Journal of Biological Chemistry* 2006;281:34610–34616. [PubMed: 16954221]
89. Sui J, Li W, Roberts A, et al. Evaluation of human monoclonal antibody 80R for immunoprophylaxis of severe acute respiratory syndrome by an animal study, epitope mapping, and analysis of spike variants. *J Virol* 2005;79:5900–5906. [PubMed: 15857975]

90. ter Meulen J, Bakker AB, van den Brink EN, et al. Human monoclonal antibody as prophylaxis for SARS coronavirus infection in ferrets. *Lancet* 2004;363:2139–2141.2141 [PubMed: 15220038] Demonstration of efficacy of an anti-SCV human monoclonal antibody in the ferret model of SCV infections.
91. van den Brink EN, ter Meulen J, Cox F, et al. Molecular and biological characterization of human monoclonal antibodies binding to the spike and nucleocapsid proteins of severe acute respiratory syndrome coronavirus. *J Virol* 2005;79:1635–1644. [PubMed: 15650189]
92. Ter MJ, van den Brink EN, Poon LL, et al. Human monoclonal antibody combination against SARS coronavirus: synergy and coverage of escape mutants. *PLoS Med* 2006;3:e237. [PubMed: 16796401]
93. Greenough TC, Babcock GJ, Roberts A, et al. Development and characterization of a severe acute respiratory syndrome-associated coronavirus-neutralizing human monoclonal antibody that provides effective immunoprophylaxis in mice. *J Infect Dis* 2005;191:507–514. [PubMed: 15655773]
94. Prabakaran P, Gan J, Feng Y, et al. Structure of severe acute respiratory syndrome coronavirus receptor-binding domain complexed with neutralizing antibody. *J Biol Chem* 2006;281:15829–15836.15836 [PubMed: 16597622] Structural insights of m396's broad neutralizing activity against various SCV isolates.
95. Xiao X, Chakraborti S, Dimitrov AS, et al. The SARS-CoV S glycoprotein: expression and functional characterization. *Biochem Biophys Res Commun* 2003;312:1159–1164. [PubMed: 14651994]
96. Chakraborti S, Prabakaran P, Xiao X, Dimitrov DS. The SARS coronavirus S glycoprotein receptor binding domain: fine mapping and functional characterization. *Virol J* 2005;2:73. [PubMed: 16122388]
97. Wong SK, Li W, Moore MJ, et al. A 193-amino acid fragment of the SARS coronavirus S protein efficiently binds angiotensin-converting enzyme 2. *J Biol Chem* 2004;279:3197–3201. [PubMed: 14670965]
98. He Y, Zhou Y, Liu S, et al. Receptor-binding domain of SARS-CoV spike protein induces highly potent neutralizing antibodies: implication for developing subunit vaccine. *Biochem Biophys Res Commun* 2004;324:773–781.781 [PubMed: 15474494] First demonstration of the potent immunogenicity of the receptor-binding domain of the SCV.
99. Jiang S, He Y, Liu S. SARS vaccine development. *Emerg Infect Dis* 2005;11:1016–1020. [PubMed: 16022774]
100. He Y, Lu H, Siddiqui P, et al. Receptor-binding domain of severe acute respiratory syndrome coronavirus spike protein contains multiple conformation-dependent epitopes that induce highly potent neutralizing antibodies. *J Immunol* 2005;174:4908–4915. [PubMed: 15814718]
101. He Y, Zhu Q, Liu S, et al. Identification of a critical neutralization determinant of severe acute respiratory syndrome (SARS)-associated coronavirus: importance for designing SARS vaccines. *Virology* 2005;334:74–82. [PubMed: 15749124]
102. Chen Z, Zhang L, Qin C, et al. Recombinant modified vaccinia virus Ankara expressing the spike glycoprotein of severe acute respiratory syndrome coronavirus induces protective neutralizing antibodies primarily targeting the receptor binding region. *J Virol* 2005;79:2678–2688. [PubMed: 15708987]
103. Yi CE, Ba L, Zhang L, et al. Single amino acid substitutions in the severe acute respiratory syndrome coronavirus spike glycoprotein determine viral entry and immunogenicity of a major neutralizing domain. *J Virol* 2005;79:11638–11646. [PubMed: 16140741]
104. Zhu ZY, Chakraborti S, He Y, et al. Potent cross-reactive neutralization of SARS coronavirus isolates by human monoclonal antibodies. *Proceedings of the National Academy of Sciences of the United States of America* 2007;104:12123–12128. [PubMed: 17620608]
105. Duan J, Yan X, Guo X, et al. A human SARS-CoV neutralizing antibody against epitope on S2 protein. *Biochem Biophys Res Commun* 2005;333:186–193. [PubMed: 15939399]
106. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* 2003;426:450–454.454 [PubMed: 14647384] Identification of the receptor for SARS coronavirus.
107. Li W, Shi Z, Yu M, et al. Bats are natural reservoirs of SARS-like coronaviruses. *Science* 2005;310:676–679.679 [PubMed: 16195424] Identification of the natural reservoir of the SCV.

108. Lau SK, Woo PC, Li KS, et al. Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats. *Proc Natl Acad Sci U S A* 2005;102:14040–14045.14045 [PubMed: 16169905] Identification of the natural reservoir of the SCV.
109. Li F, Li W, Farzan M, Harrison SC. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. *Science* 2005;309:1864–1868.1868 [PubMed: 16166518] Elucidation of the SCV S protein RBD and receptor ACE-2 complex structure.
110. Zhu Z, Dimitrov AS, Bossart KN, et al. Potent neutralization of Hendra and Nipah viruses by human monoclonal antibodies. *J Virol* 2006;80:891–899.899 [PubMed: 16378991] First identified human monoclonal antibodies against Hendra and Nipah viruses; they potently neutralize infectious virus.
111. Bonaparte MI, Dimitrov AS, Bossart KN, et al. Ephrin-B2 ligand is a functional receptor for Hendra virus and Nipah virus. *Proc Natl Acad Sci U S A* 2005;102:10652–10657.10657 [PubMed: 15998730] Identification of Hendra and Nipah viruses receptor.
112. Negrete OA, Levroney EL, Aguilar HC, et al. EphrinB2 is the entry receptor for Nipah virus, an emergent deadly paramyxovirus. *Nature* 2005;436:401–405.405 [PubMed: 16007075] Identification of Hendra and Nipah viruses receptor.
113. Xu K, Rajashankar KR, Chan YP, et al. Host cell recognition by the henipaviruses: Crystal structures of the Nipah G attachment glycoprotein and its complex with ephrin-B3. *Proceedings of the National Academy of Sciences of the United States of America* 2008;105:9953–9958. [PubMed: 18632560]
114. Bowden TA, Aricescu AR, Gilbert RJC, et al. Structural basis of Nipah and Hendra virus attachment to their cell-surface receptor ephrin-B2. *Nature Structural & Molecular Biology* 2008;15:567–572.
115. Kwong PD, Wyatt R, Robinson J, et al. Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. *Nature* 1998;393:648–659.659 [PubMed: 9641677] The first crystal structure of HIV gp120 in complex with the CD4 receptor and an antibody.
116. Zhou TQ, Xu L, Dey B, et al. Structural definition of a conserved neutralization epitope on HIV-1 gp120. *Nature* 2007;445:732–737. [PubMed: 17301785]
117. Zhu Z, Bossart KN, Bishop KA, et al. Exceptionally potent cross-reactive neutralization of Nipah and Hendra viruses by a human monoclonal antibody. *Journal Of Infectious Diseases* 2008;197:846–853. [PubMed: 18271743]

Figure 1. Crystal structures of S RBD-receptor and S RBD-antibody complexes

A. S RBD (green) in complex of the human ACE2 receptor (magenta). **B.** S RBD-Fab m396 antibody (blue-heavy chain and cyan-light chain) complex. **C.** S RBD-sc Fv 80R antibody (orange-heavy chain and yellow-light chain) complex. S RBD complex structures are projected in the same orientation with respect to the S RBD (green). The receptor and antibody binding sites on the S RBD are colored in red. **D.** A close-up view of the S RBD-ACE2 interface. Tyr491 of $\beta 6$ - $\beta 7$ loop from S RBD makes a key contact with Lys353 of ACE2. **E.** Tyr491 of S RBD is involved in the critical interaction with Fab m396 antibody through cation-pi and hydrogen bond interactions, respectively, with Thr52 and Asn58 of CDR H2. **F.** A close-up view showing the S RBD-sc Fv 80R antibody interface where Tyr491 of S RBD interacts with Asp99, Arg100 and Ser101 of CDR H3, and Tyr32 of CDR H1.

Figure 2. Comparison of the complex structures of Nipah and Hendra attachment G glycoproteins, and HIV-1 gp120 glycoprotein

A. NiV G – ephrin-B3 complex (NiV G in magenta and ephrin-B3 in yellow) showing the dominant virus-receptor interaction involving Tyr120 in the G–H loop of ephrin-B3 which snugly fits into the hydrophobic pocket as shown in gray on the surface portion of NiV G glycoprotein. **B.** HeV G – ephrin-B2 complex (HeV G in green and ephrin-B2 in cyan) in which Phe120 in the G–H ephrin loop inserts into a channel shown in red on the surface of NiV G. **C.** HIV gp120 glycoprotein (red) complex with its receptor CD4 (yellow) extracted from the HIV gp120-CD4-17b ternary complex (D1 domain of CD4 and 17b antibody are not shown). Phe43 of C'C" loop in CD4 makes a critical contact with gp120, and reaches the hydrophobic pocket, shown as gray surface, formed in between the inner and outer domains of gp120. **D.** HIV-1 gp120 (red) in complex with the b12 antibody (partly shown, heavy chain in blue, light chain in pink). Tyr53 from the CDR H2 loop of b12 antibody fits into the hydrophobic pocket on the gp120 surface (gray) in a similar manner as “Phe43” of CD4 receptor interacts with the gp120.

Table 1
Potent neutralizing human monoclonal antibodies against the SARS CoV S glycoprotein.

Antibody	Source	Specificity	Animal model	Remarks and Reference
S3.1	EBV transformation	S protein	Mouse	Inhibited entry of pseudovirus with S protein from the Urbani isolate but not the GD03T0013 isolate. [85, 86]
S215.13	EBV transformation	S protein	Mouse	300-fold lower neutralizing concentration than that of S3.1. [85]
80R	Phage display	S1 domain	Mouse	Bound S1 with nanomolar affinity and blocked the binding of S1 to ACE2. X-ray structure of 80R scFv – SARS CoV RBD complex is known. Epitope is sparse and overlapping with the ACE2 binding site. [87,88]
CR3014	Phage display	S1 domain	Ferrets	Asn479 of S1 domain is critical for antibody binding. All neutralization escape mutants carried a point mutation Pro462Leu. [90,91,92]
CR3022	Phage display	S1 domain	None	Neutralized CR3014 escape mutants. CR3014 and CR3022 neutralized SARS-CoV synergistically. [92]
201	Transgenic mice	S protein	Mouse	Bound the amino acids 490–510 and could interfere with the ACE2 binding. [93]
68	Transgenic mice	S protein	Mouse	Bound the amino acids 130–150 and did not interfere with the ACE2 binding. [93]
m396	Phage display	S1 domain	Mouse	Potently inhibited S-mediated fusion with an IC ₅₀ of 0.6 µg/ml. X-ray structure of m396 Fab – SARS CoV RBD complex is known. Epitope lies mostly within a loop and overlaps with the ACE2 binding site. [94, 104]
S230.15	EBV transformation	S1 domain	Mouse	Both m396 and S230.15 exhibit potent inhibitory activity against isolates from the first outbreak as well as the second one and from palm civets. [104]
B1	Phage display	S2 domain	None	Epitope was located on S2 domain within amino acids 1023–1189. [105]