Chapter 33
Human Monoclonal Antibodies Against HIV and Emerging Viruses

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33.1 Introduction

Throughout the centuries, viral diseases have killed hundreds of millions of people and continue to do so. More than 100 million died in the past from smallpox caused by the variola virus. Influenza killed about 50 million people during the 1918 pandemic. More than 60 million have been infected with HIV since the first cases of the epidemic were reported in 1981, and more than 20 million have died from AIDS. Although vaccines remain the most cost-effective way to prevent viral infections, in many cases (e.g., HIV) vaccines are not available and, in other cases, available vaccines have to be modified frequently to counteract virus escape (e.g., influenza). Prophylaxis and treatment in such cases are critically important to fight viruses.

A number of studies have shown the importance of neutralizing antibodies in recovery and protection from viral infections *(1, 2)*. Sera from humans or animals containing antibodies 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 (Ig) has continued to be also used for viral diseases where there are few treatments available, although mostly for prophylaxis either prior to an anticipated exposure or following an exposure to an infectious agent *(7–9)*. Antibody products licensed in the United States for prevention or treatment of viral diseases include human Ig for use against hepatitis A and measles; virus-specific polyclonal human Ig against cytomegalovirus, hepatitis B, rabies, respiratory syncytial virus (RSV), vaccinia, and varicella-zoster virus; and the humanized monoclonal antibody (mAb) Synagis *(7)*. Polyclonal Ig has also been used with various success for diseases caused by other human viruses including parvovirus B19 *(10–13)*, Lassa virus *(14, 15)*, West Nile virus *(16, 17)*, some enteroviruses *(18, 19)*, herpes simplex virus *(20)*, Crimean-Congo hemorrhagic fever virus *(21)*, Junin virus *(22)*, SARS-CoV *(23, 24)*, and 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)*. mAbs including chimeric animal-human, humanized, and fully human mAbs (hmAbs) possess lower or absent immunogenicity, toxicity, and lot-to-lot variation. 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 (palivizumab), which is still the only mAb against a viral disease approved for clinical use by the U.S. Food and Drug Administration, 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; for example, there were no significant differences in the clinical outcomes between the placebo and the palivizumab groups for children hospitalized with RSV infection *(32)*; in addition, resistance can develop relatively quickly: a recent study found F gene-resistant mutations in an animal model of the RSV infection (cotton rat) 12 weeks after infection including a completely resistant virus *(33)*.

The development of hmAbs for prophylaxis and treatment of diseases caused by HIV and emerging viruses is still in an initial stage. This chapter reviews hmAbs with potential for prophylaxis and treatment of diseases caused by HIV-1, SARS-CoV, and henipaviruses (Hendra [HeV]) and Nipah [NiV]). Mostly IgG_1 are discussed unless specifically noted otherwise; other isotypes also could be useful but are not so frequently used and other formats including Fabs and single-chain variable region fragments (scFvs) are noted when described. Finally, possible implications for development of effective vaccines are discussed.

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33.2 HIV

The demand for new treatment options against HIV is becoming increasingly important as the side effects and the expansion and spread of drug-resistant virus within the infected population limit the clinical benefits provided by available anti-HIV drugs. Despite the promise presented by Synagis and its improved variants *(31)*, developing effective antibody-based therapeutics against HIV presents an especially difficult challenge. Therapies based on small molecule-based drugs eventually fail because of the expansion of a drug-resistant virus population in the infected individual. Antibody-based therapies are not immune to this problem. Further, HIV-1 replicates and spreads within the densely packed cellular environment (reaching about 10^8 cells per mL) of the lymphoid tissues of the gut, spleen, and lymph nodes. Antibodies may have difficulty preventing the cell-to-cell spread of virus in this seemingly impenetrable lymphoid environment. In fact, passive administration of anti-HIV antibodies as human immune plasma or polyclonal antibody preparations conferred, at best, only modest clinical impact *(8, 29)*. Unfortunately, these trials were complicated by the relative absence of highly effective concentrations of HIV-neutralizing activity in the polyclonal preparations used. Preparations containing high concentrations of hmAbs that exhibited potent HIV-1-neutralizing activity *in vitro* (nhmAbs), on the other hand, resulted in measurable decrease of plasma virus concentration *(34)*. However, the *in vivo* potency of this preparation was insufficient to completely block virus replication, and resistant virus rapidly emerged. Despite this discouraging result, these studies suggest that antibodies with enhanced *in vivo* potency could have a more profound clinical impact.

A major question is whether antibody-based therapeutics can provide long-term clinical benefits for patients with established infections that are comparable to or better than drugs currently in clinical use. A specific feature of antibodies compared to other drugs is that HIV has evolved a number of strategies to escape neutralization. Such evasion strategies of the virus against polyclonal antibodies elicited during infection and strategies used by the immune system to generate broadly neutralizing hmAbs have been extensively reviewed *(1, 35–40)*. Thus, a short answer to this question is perhaps it is possible if we can outsmart the virus by engineering potent antibody-based therapeutics against which the virus has not yet evolved protective strategies. Here, we discuss our ongoing efforts to improve the potential clinical utility of already known hmAbs and identify novel, more potent antibodies against HIV.

33.2.1 Anti-HIV Antibodies Elicited by Infection or Immunization

HIV entry into cells is initiated by attachment of the viral envelope glycoprotein (Env) to a host cell receptor (CD4). Conformational changes follow, which enable enhanced exposure of a co-receptor (typically CCR5 or CXCR4) binding site and binding of the viral glycoprotein gp120 to the co-receptor. Subsequent conformational changes finally result in fusion of the viral and cell membranes. In some cases, CD4 is not required and the Env directly interacts with a co-receptor. Because the Env mediates HIV entry and is the only viral surface protein exposed to the surrounding environment, it is a major target for neutralizing antibodies and a potent immunogen *(36)*. Env-specific antibodies are generated as early as a few weeks after productive infection or immunization. They do not typically neutralize current virus isolates but rather neutralize earlier isolates *(41)*. Such antibodies are isolate-specific and lack broad neutralizing activity because the virus has evolved to hide conserved epitopes and escape neutralization by a number of mechanisms. As a result, most of the antibodies generated in natural infection or immunization are non-neutralizing or neutralize few isolates. More than 100 mAbs have been reported as recognizing epitopes on gp120 and gp41, but only a small number exhibit neutralizing activity against primary isolates from different clades, denoted as broadly cross-reactive neutralizing hmAbs (bcnhmAbs).

33.2.2 HIV-1-neutralizing hmAbs Against the Env

Using phage display or B cell immortalization, several bcnhmAbs were identified from HIV-infected patients whose sera contained a high titer of such antibodies. Six major classes of such antibodies relevant to the binding location and properties of their epitopes have been identified: (1) antibodies that bind to the region containing the CD4 binding site (CD4bs) on gp120; (2) antibodies binding better to gp120 complexed with CD4 than to gp120 alone (CD4i antibodies); (3) carbohydrate-binding antibodies; (4) gp120 V2 or V3-binding antibodies; (5) gp41 antibodies targeting the membrane-proximal external region (MPER); and (6) antibodies binding to other epitopes on gp41.

The best characterized and very potent CD4bs antibody is b12, a hmAb selected from a phage-displayed antibody library constructed from the bone marrow of an HIV-1-infected donor *(42, 43)*. The CD4 binding site is masked by V1/V2 variable loops and further shielded by *N*-glycan in the region. The limited accessibility of these conserved epitopes in the context of the oligomeric Env could explain why most of the antibodies that are specific for the CD4 binding site bind weakly to Env oligomers and exhibit weak to moderate neutralizing activities against primary HIV-1 isolates. Recently, two novel nmAbs (m14, m18) were identified by sequential antigen panning (SAP) against purified Env ectodomains (gp140s) of another phage-displayed antibody library derived from the bone marrow of three long-term nonprogressors with high titers of bcnhAbs *(44, 45)*. These antibodies cross-react with Envs from primary isolates and exhibit differential neutralizing activity with b12 to isolates from different clades.

The Env undergoes significant conformational changes after binding to CD4 leading to exposure of structures that contain epitopes or portions of epitopes targeted by CD4i antibodies. The best characterized CD4i antibody, 17b, is only weakly neutralizing as IgG1, but its neutralizing activity increases significantly after its size is reduced to a scFv *(46)*. The neutralizing activity of the most potent and broadly neutralizing CD4i antibody, X5 *(47)*, also increases in many cases with decreasing its size to scFv or Fab. One should note however, that for some isolates and in some assays including assays based on spreading infection in peripheral blood mononuclear cells (PBMCs), IgG1 X5 is more potent than Fab X5 likely due to an increase in avidity. Thus, an interplay between avidity and size could be important in determining the neutralizing activity of CD4i antibodies.

Many gp41-specific antibodies have been identified of which three with linear epitopes, 2F5, 4E10, and Z13, exhibit broad neutralizing activity and have been extensively characterized *(48)*. They can bind peptides containing stretches of the MPER of gp41, which is relatively conserved. Using a competitive antigen panning methodology, several new cross-clade reactive anti-gp41 mAbs have been identified (m43, m44, m45, m46, m47, and m48) that neutralize a variety of primary isolates in PBMC/infectious virus-based assays and bind to conformational epitopes distinct from those of other anti-gp41 antibodies *(49)*.

33.2.3 Evidence That Antibodies Can Affect HIV-1 Replication in Humans

There are several lines of evidence that antibodies can inhibit HIV-1 infections. First, it has been demonstrated that antibodies can exert selective pressure in humans suggesting that they inhibit infection *(41)*. Second, HIV-1 concentration in plasma of patents decreases following administration of hmAbs *(34)*. Third, a triple combination (2G12, 2F5, 4E10) of nmAbs at higher doses can delay viral rebound after cessation of antiretroviral treatment *(50)*. In addition, a number of experiments have shown that antibodies can prevent infection in monkeys (see ref. *37* for a recent review).

33.2.4 Developing Antibodies With Improved Neutralizing Activity

Naturally occurring antibodies with potent and broad neutralizing activity could be further improved. Recently, scFv X5 was further improved in potency and breadth of neutralization by using the SAP methodology and a library of X5 mutants; two scFvs, m6 and m9, which exhibited on average two- to three-fold lower IC_{50} than scFv X5 were identified *(44)*. The scFv X5 mutant library was also screened by surface plasmon resonance technique on immobilized Envs. One scFv was selected on the basis of its higher kinetic association rate, and its activity is being evaluated. Because CD4i epitopes may be available for limited time after CD4 binding to gp120 and before their interaction with a co-receptor, CD4i antibodies with faster binding kinetics would bind efficiently and are likely to have better inhibitory activity against HIV-1. We have been developing various other constructs including fusion proteins that are being evaluated for neutralizing activity.

33.2.5 Conclusions (HIV)

HIV has evolved a number of strategies to escape host immune surveillance, prominently by modifications of its Env. Thus, naturally occurring whole antibodies against its Env may have little chance of significantly affecting viral replication and disease progression, as also evidenced by the lack of sustained significant effect in the few clinical trials that have tested such antibodies. This is mostly due to the rapid generation of resistant mutants, which remains a fundamental problem not only for antibodies but also for other antiretroviral drugs. Antibodies against components of the entry machinery, engineered antibody fragments and their derivatives, and other antibody-based inhibitors that do not occur naturally and against which the virus has not developed defense mechanisms may be better able to control virus replication, although mechanisms of inhibitory escape such as generation of resistant mutants and difficult access could still be operating. The challenge is to fight these mechanisms and simultaneously ensure a relatively long half-life and biological effector functions. Several directions of research appear promising in this aspect. One direction involves engineering antibody with higher binding affinity to conserved epitopes and with smaller size than the currently known antibodies. Another uses the unique features of some anti-Env antibodies (e.g., the mimicry of receptors) to engineer novel binding entities with high-binding affinity to many isolates. A third consists of engineering novel fusion proteins containing antibody-binding fragments. A fourth relies on using antibody effector functions, including antibody-dependent cellular cytotoxicity (ADCC) and complement, to increase the efficacy *in vivo*. Yet another direction of research aims to develop antibody-nanoparticle conjugates, or nanoliposomes in particular, that are able to irreversibly inactivate virus and cells expressing viral proteins. The development of novel approaches may also be fruitful in the future, as well as combining existing ones to develop antibody-based HIV inhibitors using other, yet undiscovered principles. Whether these or other research directions will lead to clinically useful antibody-based inhibitors of HIV infections remains unknown; however, even if new engineered antibodies fail to inhibit HIV infections in a clinically useful way, there is still a basis for optimism in this endeavor because the new approaches can prove useful elsewhere. Finally, one should note that all bcnhmAbs have undergone a long maturation process and differ from the germline sequences by tens of mutations. One could speculate that generation of such antibodies *in vivo* following immunization is very unlikely because of the lack of B cells expressing surface-associated Ig that is close in function to those bcnhmAbs. This may represent

a challenge in developing effective AIDS vaccines, and further studies are required to find novel approaches for elicitation of bcnhmAbs *in vivo*.

33.3 SARS-CoV

The SARS-CoV *(51–54)* caused a worldwide epidemic in 2002 and 2003, and infected more than 8,000 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 infection leads to generation of potent neutralizing antibodies that can affect the course of infection and help clear the virus; they can also protect an uninfected host exposed to the virus. Antibodies that neutralize the virus in *in vitro* assays were detected in SARS-CoV-infected patients *(55–60)*, and in mice *(61)*, hamsters *(62)*, and monkeys *(63)* 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 *(61)*. Patients infected with SARS-CoV were also treated with convalescent patient plasma containing polyclonal antibodies *(64, 65)*, improvements of the antibody preparations were suggested *(24)*, and batches of virus-inactivated hyperimmune globulins containing five to six times higher titers of SARS-CoV-specific antibodies than convalescent plasma were produced *(66)*. In an amazing pace of research, several groups have recently developed hmAbs to the SARS-CoV spike (S) glycoprotein that neutralize the virus and have potential for therapy and prophylaxis of SARS (reviewed in ref. *67*).

Recently, an improved method for Epstein-Barr virus 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 used for selection of hmAbs specific for SARS-CoV proteins *(68)*. 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 *(68)*, and inhibited entry of pseudovirus with S glycoprotein from Urbani isolate with about the same IC_{50}. However, it did not affect to any significant extent pseudovirus entry mediated by the GD03 isolate S glycoprotein and even enhanced the entry of virus pseudotyped with the S glycoprotein from the palm civet isolate SZ16 *(69)*. In a mouse model of SARS-CoV infection, this antibody prevented viral replication in the lower respiratory tract (at doses of 200 and 800 μg), and reduced it in the upper respiratory tract at the highest dose (800 μg) used. Unfortunately, data for the *in vivo* neutralizing activity of other nhmAbs selected in this study *(68)*, 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. 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 through 672 *(70)*. 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 *(70)*. When 80R IgG1 was given prophylactically to mice at doses therapeutically achievable in humans, viral replication was reduced to below assay limits *(71)*. 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 previously and later; 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 *(72, 73)*. They bound a recombinant S1 fragment comprising amino acid residues 318 to 510 that also binds the receptor ACE2—the receptor-binding domain (RBD; ref. *73*). The most potent of these hnmAb, IgG1 CR3014, required the residue N479 for binding *(73)*. 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 $TCID_{50}$; high: 10^4 $TCID_{50}$) or with virus preincubated with the antibody at 0.13 mg/mL for the low dose and 1.3 mg/mL for the high dose. 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 hours 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. These results suggest a potential use of CR3014 for prophylaxis of SARS-CoV infections in humans if it can reduce the virus replication to the same extent as in ferrets. 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 *(74)*. One of these antibodies [201] bound within the RBD of the S protein at amino acid residues 490 through 510, and the other one *(68)* to a region including residues 130 through 150. In a microneutralization assay based on protection to cytopathic effects the IC_{50} for 201 was about 0.2 μg/mL *(74)*. 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, and further studies are planned to evaluate the nhmAb 201 for potential clinical use *(74)*.

We have recently identified a novel cross-reactive potent SARS-CoV-neutralizing hmAb, m396, 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 *(75)*. This fragment was previously identified to contain the RBD *(76–78)*, which is a major SARS-CoV neutralization determinant *(67, 79–84)*. It potently inhibited S-mediated cell fusion (IC_{50} = 0.6 μg/mL), pseudovirus entry (IC_{50} = 0.01 μg/mL), and replication of infectious virus in mice (complete protection at 0.2 mg per mouse; Zhu et al., unpublished). Interestingly, this antibody also inhibited entry mediated by the S glycoprotein from the 2003/2004 GD03 isolate, which has a 487Thr/Ser mutation compared to the middle/late phase 2002/2003 isolate Tor2, and is not neutralizable by other known potent hmAbs including 80R and S3.1. It bound with high (pM) avidity to the RBD in a Biacore chip and competed with ACE2 suggesting a mechanism of neutralization that involves competition with the receptor for binding to the S glycoprotein (Zhu et al., unpublished). The epitope of this antibody was identified by crystallography and proposed as a possible vaccine immunogen (a retrovaccinology approach for design of vaccine immunogens [*85*]).

Neutralizing antibodies directed to S inhibit SARS-CoV entry either by interfering typically with the S RBD-receptor interactions *(70)* or by other mechanisms including binding to other portions of S. Recently, a human scFv, B1, was identified, which recognizes an epitope on S2 protein located within amino acids 1023 to 1189 *(86)*. 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 are not discussed here) are related to the antibody biological effector functions conferred by the antibody Fc, e.g., ADCC.

33.3.1 NiV and HeV

NiV and HeV are closely related emerging paramyxoviruses that comprise the *Henipavirus* genus *(87–96)*. 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 ref. *1*). They are Biological Safety Level 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, the development of hmAbs specific for HeV and NiV have only just been realized.

We recently reported 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 *(97)*. 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 IC_{50} 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. M101 and m102 competed with the ephrin-B2, which we and others recently identified as a functional receptor for both HeV and NiV *(98, 99)*, indicating a possible mechanism of neutralization by these antibodies. The m101, m102, and m103 antibodies competed with each other suggesting that they bind to overlapping epitopes that are distinct from the epitopes of m106 and m107. In an initial attempt to localize the epitopes of m101 and m102, we measured their binding to a panel of 11 G alanine scanning mutants and identified two mutants, P185A and Q191,K192A, which significantly decreased binding to m101, and one, G183A, which decreased binding of m102 to G. These results suggest that m101-7 are specific for HeV or NiV or both, and exhibit various neutralizing activities; they are the first hmAbs identified against these viruses and could be used for treatment, prophylaxis, diagnosis, and as research reagents and aid in the development of vaccine.

Recently, we matured *in vitro* m102 to a very potent cross-reactive antibody, m102.4, which neutralized both infectious NiV and HeV with an IC_{50} of 100 ng/mL (Zhu et al., unpublished). We developed a cell line that produces large amounts of IgG1 m102.4. This antibody will be tested in an animal model of henipavirus infection and if successful, which is very likely, it could be clinically useful for humans.

Interestingly, the antibodies against SARS-CoV and henipaviruses were identified from naïve libraries, and in contrast to HIV have only few mutations compared to the respective germline sequences. This correlates with the ability of various vaccines to elicit potent cross-reactive neutralizing antibodies against these viruses in contrast to HIV. One could speculate that humans have in their native repertoire antibodies that can bind with relatively high affinity envelope glycoproteins from these emerging viruses but not to the HIV Env. Further experiments are required to test this hypothesis, which if true could lead to novel approaches for design of effective vaccines against HIV and other viruses.

33.3.2 Conclusions (SARS-CoV and Henipaviruses)

The hmAbs directed to the SARS-CoV S glycoprotein and the henipavirus G glycoprotein are currently in an 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 a clinically useful product for prophylaxis and perhaps treatment of the diseases caused by these infections. They are very potent, and the viral infections to which they are specific are acute, such that only control or dampening of virus replication for a relatively short period of time (few weeks) is likely to be required after which the host immune system could control virus replication. In addition, these antibodies could be cross-reactive. 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 a high level of virus replication, e.g. HIV infections. A note of caution is that careful examination of candidate antibody therapeutics is required because of the possibility for infection enhancing effects, as e.g., for Ebola, 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 *(69)*. 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 tested also for their effector functions mediated by the Fc including antibody-dependent direct cytotoxicity 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.

The rapid progress made in the last few years toward the development of potent neutralizing hmAbs against emerging viruses and viruses of biodefense importance is a basis for further more accelerated development of neutralizing antibodies in the next 5 years and their testing in animal models. It is likely to see novel and even more potent antibodies against the SARS-CoV than the currently existing ones. They could be used alone or in combination with the existing antibodies in animal models of viral diseases and for evaluation of toxicity in human clinical trials. The currently available hmAbs against NiV and HeV are likely to be tested in animals. If successful, which is very likely, they can undergo evaluation in human clinical trials. Although the interest of big- and medium-size companies to such antibodies appears to be relatively minor, small companies and start-ups could be interested in developing such antibodies provided there is a continued governmental support by programs like Bioshield. Five years from now, it is likely to have at least several hmAbs of potential clinical use in case of outbreaks or terror attacks. These antibodies could be used in combination with other therapeutics to increase potency and cope with resistance. Several key issues are listed below:

- Continuation of research and development funding at the same or accelerated pace is of critical importance for development of potent and clinically useful therapeutic antibodies.
- Phage display techniques as well as novel methodologies will be critical for the development of fully human antibodies.
- Cloning, expression, and purification of novel antigens for screening of human antibody libraries is of critical importance.
- Crystal structures of antibody complexes with virus envelope glycoproteins and their use for further improvement of the antibodies and understanding of their interactions is of critical importance.
- Development of appropriate novel animal models that would be of critical importance for the accelerated testing of the therapeutic antibodies is necessary.
- Understanding of the pathogenesis and the design of antibodies acting through multiple mechanisms with an increased efficacy *in vivo* is of critical importance.
- Evaluation of combinations of antibodies and other antiviral drugs is of critical importance.

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