CME Article

The use of antibodies in the treatment of infectious diseases

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ABSTRACT

There is a long history of the use of antibodies in the treatment and prophylaxis of infectious diseases, because these molecules play a critical role in directing the effector mechanisms of the immune system against the pathogens they recognise. However, the widespread application of this therapy has been hampered by allergic reactions, production costs and the availability of alternative drugs such as antibiotics. Some of these obstacles can now be overcome with advances in biotechnology, which has enabled the development of antibody-based drugs for use first in treating cancer, and recently, for treating infectious diseases. The efficacy of such antibodies has been demonstrated in various *in vitro* studies, animal models and clinical trials for a variety of both viral and bacterial pathogens. Antibodies appear to hold great promise as a new class of drugs against infectious diseases.

Keywords: immunotherapy, monoclonal antibody, passive immunity, therapeutic antibodies

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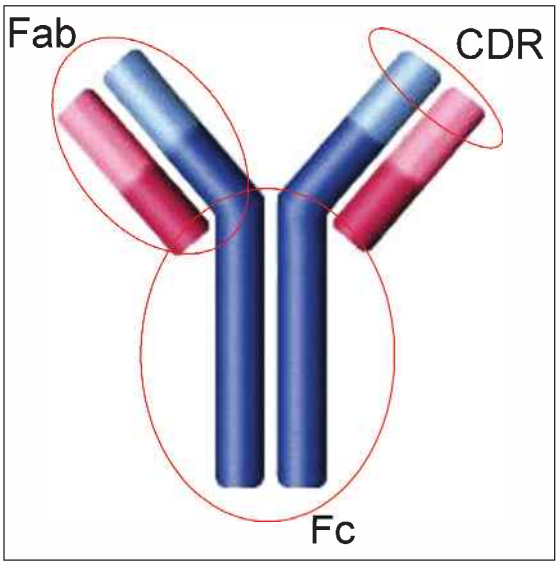

Fig. 1 Schematic diagram shows the antibody structure. Two heavy chains (blue) and two light chains (red) comprise the full antibody. The lighter and darker shading indicates the variable and constant regions respectively. The CDR is located at the tip of each Fab. Fab: antigen-binding fragment; CDR: complementarity-determining region; Fc: fragment crystallisable

INTRODUCTION

Antibodies are the workhorses of the humoral immune response, providing both target recognition and the signal for effector functions. As such, antibodies were once the treatment of choice for certain life-threatening infectious diseases until the advent of antibiotic therapy. However, with the increasing prevalence of antimicrobial resistance as well as the emergence of newly-recognised infectious diseases, the use of therapeutic antibodies for infectious diseases could take on a new significance. We review the history and the current progress in the development of this field for use in infectious diseases therapeutics.

BRIEF OVERVIEW OF ANTIBODIES

Human antibodies consist of four polypeptide chains, two pairs of heavy and light chains in a Y-shaped arrangement (Fig. 1). The two arms of the Y are created by the pairing of heavy and light chains, forming the antigen-binding region which provides for target recognition. The unpaired sections of the longer heavy chains interact to form the tail of the Y, linked to the arms by a flexible linker or "hinge". This tail section, termed the fragment crystallisable (Fc) region, is common to all antibodies of the same type and serves to provide the signal for effector functions. The antigen-binding domain, also called the fragment variable (Fv) region, binds the antigen through interaction with the surface formed by six complementarity-determining regions (CDRs), three each from the heavy and light chains located at the tip of the domain. It is the amino acid sequence of the CDRs that varies between individual antibodies and that is primarily responsible for determining the specificity of each unique antibody. Therefore, through genetic recombination, the humoral immune response is capable of generating an antibody repertoire of immense diversity in the CDR region, and hence, has the ability to bind the wide range of antigens found on pathogens.

Antibody molecules mediate their protective effects through the recognition of specific antigens on their target pathogens. For some protective effects, such as

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virus and toxin neutralisation, binding of the antibody alone provides sufficient steric interference to disrupt the interaction between the given antigen and its cellular receptor, thereby abrogating virus uptake and replication or intoxication. However, other protective effects, such as phagocytosis, complement activation or antibody-dependent cellular cytotoxicity (ADCC), depend on the bound antibody recruiting other components of the immune system. This occurs through the binding of complement proteins in serum or Fc receptors (FcR) on the surface of immune cells to the Fc region of the antibody. More recently, antibodies have been characterised that have direct antifungal or antibacterial action *in vitro*.[1,2] In addition, it has been proposed that antibodies play additional roles in regulating cell-mediated immunity and inflammatory responses.[3]

ANTIBODY THERAPY IN THE PRE-ANTIBIOTIC ERA

With the discovery of the protective properties of sera by von Behring and Kitasato in 1890, the administration of hyperimmune sera from immunised animals or immune human donors, termed serum therapy, was considered as an option for the treatment of infectious diseases. Indeed, in 1894, von Behring, in collaboration with others, was producing anti-diphtheria serum in dairy cattle for therapeutic use.[4] By the early 20th century, serum therapy was widely used to treat a variety of bacterial infections, such as *Streptococcus pneumoniae* and *Neisseria meningitidis*.[5] Unfortunately, this treatment often had significant side effects due to the immune response against the animal-derived antibodies, the most severe being serum sickness, a type of delayed hypersensitivity response characterised by fever, chills, rashes, arthritis, and occasionally glomerulonephritis. Furthermore, the specificity of individual antibodies meant that separate immune sera had to be raised for different pathogens, and in the case of *Streptococcus pneumoniae*, individual serotypes.[5]

Hyperimmune sera also had the disadvantage of being a polyclonal antibody preparation containing undefined concentrations of multiple specific and non-specific antibodies. As a result, it was extremely difficult to standardise serum quality and ensure the efficacy of the therapeutic serum products. These complications, together with the discovery of penicillin and the rise of the antibiotic age, brought about the abandonment of serum therapy for the treatment of bacterial infections by the early 1940s. Nevertheless, this early adoption of antibody-based therapy demonstrates the therapeutic potential of antibodies.

REVIVAL OF PASSIVE IMMUNITY: ANTIBODY TECHNOLOGY DRIVES ANTIBODY DEVELOPMENT

The last few decades have seen a revival of interest in the therapeutic potential of antibodies, and antibodies have become one of the leading classes of biotechnology-derived drugs. As of last year, 17 antibody-based drugs have been approved for use and over 160 are currently under development.[6] Altogether, they account for 20% of all biopharmaceuticals in development. Initially, the revival was driven by a search for alternatives to traditional small molecule drugs. Despite decades of intensive research on cancer and AIDS, these drugs have not succeeded in providing a definitive cure for either disease, and those that do show significant efficacy also have significant side effects. In addition, many pathogens have demonstrated a marked ability to gain resistance to antimicrobial drugs, such as methicillin-resistant *Staphylococcus* (*S.*) *aureus*, extreme drug-resistant *Mycobacterium tuberculosis* and *Plasmodium falciparum*.

In contrast to traditional drugs, antibodies have two properties that make them highly attractive as therapeutic agents. The first is their low toxicity, as antibodies are endogenous proteins native to the body. The second is their high specificity. In contrast to the shotgun approach of chemotherapy and radiotherapy, the high specificity of antibodies opens up the possibility of directed targeting, making them extremely attractive as potential cancer therapies. However, initial studies on the therapeutic use of antibodies against cancer only started in the 1960s, when researchers attempted to identify the unique surface markers of cancerous cells, which could be used as targets against which polyclonal sera could be made.[7] Unfortunately, these studies had little success due to the inherent problems associated with using sera in humans.

Hybridoma technology, first reported in 1975, was thought to be the answer to the problems facing polyclonal sera. By immortalising murine B lymphocytes through fusion with a myeloma cell for the first time, it was now possible to produce individual high-affinity, specific antibodies, termed monoclonal antibodies (mAbs), continuously.[8] Unfortunately, the excitement over these antibodies as therapeutics was short-lived when early clinical trials with mAbs against cancer targets showed that their administration to patients triggered an immune response against the foreign antibody and the generation of human anti-mouse antibodies.[9] Often, this resulted in allergic reactions and faster clearance of the delivered antibody, and in some cases, neutralisation of therapy and tumour escape.[10-12]

Advances in our understanding of molecular biology

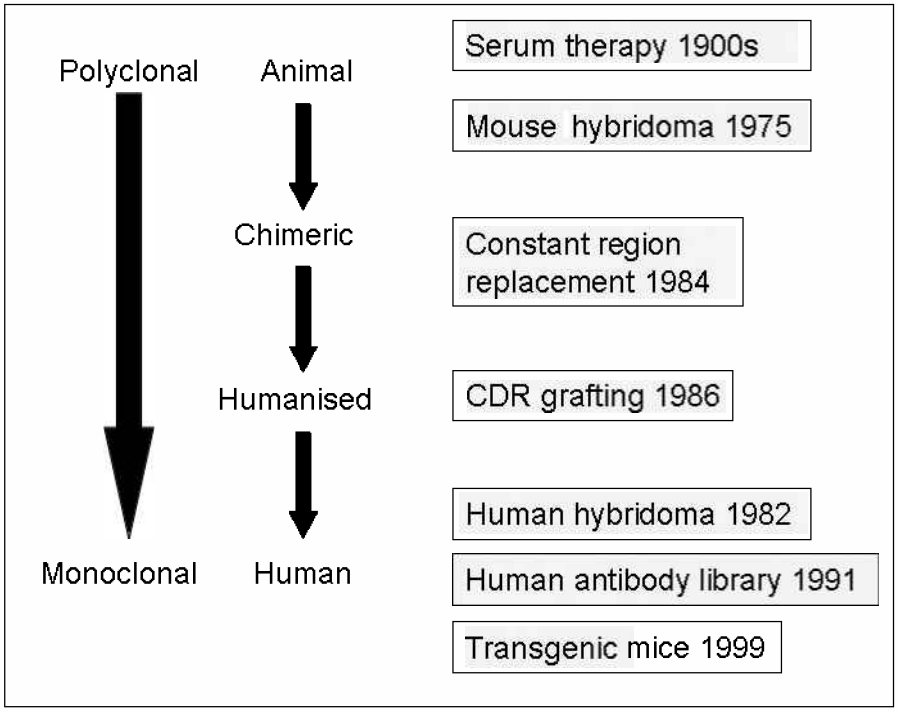

Fig. 2 Chart shows the milestones in antibody technology. Various biotechnology developments (boxes) have enabled the shift from polyclonal serum towards monoclonal and increasingly more human antibodies.

have enabled the development of genetic engineering techniques to overcome such adverse reactions, a list of which is given in Fig. 2. This is achieved by converting murine and other non-human antibodies into antibodies that would trigger a reduced or even negligible hypersensitivity response by replacing non-human regions with the corresponding human region.[13] This conversion was initially achieved by substituting the constant region of a mouse antibody with the corresponding human one.[14] Aside from reduced immunogenicity, such a chimeric antibody was able to engage more effectively the immune effector functions such as ADCC and phagocytosis. However, these chimeras still contain a completely murine variable region. In order to further eliminate the mouse sequences, CDR grafting was developed. CDR loops responsible for antigen recognition were taken from the murine antibody sequence and cloned into the respective regions of the corresponding human antibody, resulting in an almost completely human antibody with the specificity of the former murine antibody.[15] More recently, methods that take advantage of developments in computer bioinformatics have been developed to scan non-human antibodies for known human HLA class I or II epitopes. Removal of these epitopes through point mutation of specific amino acids is expected to completely abrogate detection by the immune system.[16]

The importance of reducing immunogenicity with chimeric and humanised antibodies was amply demonstrated in a meta-survey of the anti-antibody hypersensitivity responses (AAR) produced by the various murine, chimeric and humanised therapeutic antibodies in clinical trials.[17] This survey classified these responses as negligible if AAR occurred in less than 2% of patients, tolerable if AAR occurred in 2%–15% of patients and marked if AAR occurred in more than 15% of patients. Out of a total of 44 murine antibodies surveyed, 84% of these produced marked AAR in patients during clinical trials. In contrast, only 40% of the chimeric and 9% of the humanised antibodies surveyed produced marked AAR. On the other hand, 55% of humanised antibodies had a negligible AAR, dropping to 33% and 9% for chimeric and mouse antibodies, respectively.

THE DEVELOPMENT OF FULLY HUMAN MONOCLONAL ANTIBODIES

The drive to humanise animal-derived monoclonal antibodies was complemented by the development of methods for obtaining fully human monoclonal antibodies, both *in vitro* through antibody library screening and *in vivo* by the immortalisation of B cells and the development of transgenic animals (Fig. 2). An antibody library is essentially a genetic collection of different antibodies, usually the antibody repertoire that is found in one or more individuals, and can consist of up to 10^{10-11} clones.[18] Obtained by cloning out the antibody genes of B lymphocytes from human donors or using synthetic

Prefix	Infix (target)		Infix (source)		Suffix
Variable	*-vi(r)-*	viral	*-u-*	human	*-mab*
	-ba(c)-	bacterial	*-o-*	mouse	
	-fu(ng)-	fungal	*-a-*	rat	
	-le(s)-	infectious lesion	*-e-*	hamster	
	-tox(a)-	toxin as target	*-i-*	primate	
	-li(m)-	immune system	*-xi-*	chimeric	
	-ki(n)-	interleukin as target	*-zu-*	humanised	
	-ci(r)-	cardiovascular	*-axo-*	rat/murine hybrid	
	-mu(l)-	musculoskeletal	*-xizu-*	chimeric + humanised	
	-neu(r)-	nervous system			
	-o(s)-	bone			
	-co(l)-	colonic tumour			
	-me(l)-	melanoma			
	-ma(r)-	mammary tumour			
	-go(t)-	testicular tumour			
	-go(v)-	ovarian tumour			
	-pr(o)-	prostate tumour			
	-tu(m)-	miscellaneous tumour			

Fig. 3 List shows the nomenclature of monoclonal antibodies. Nonproprietary names of monoclonal antibodies can be separated into a prefix, two infixes and one suffix. The suffix is always mab and the prefix is variable. The first infix is defined by the antibody target and the second infix by the source of the antibody. Therefore Pali-vi-zu-mab is a chimeric (zu) antibody against a virus (vi), while Ada-lim-u-mab is a fully human (u) antibody against an immune system cytokine (li), TNF-α [Adapted from Guidelines to Naming Biologics, United States Adopted Names (USAN) Council].

antibody genes made from a fixed human framework and randomly generated CDRs, these antibodies are then expressed on the surface of a suitable host, e.g. yeast, bacteria or phage (bacterial virus), as a polyclonal collection and then screened for individual monoclonal antibodies that bind the desired antigen. This method of antibody screening is termed yeast, bacteria or phage display, depending on the type of host used. Occasionally, antibody libraries are made that just consist of variants of one parent antibody, generated through mutations of the variable region. These libraries are usually used to evolve antibodies with higher affinity or broader specificity from a parent antibody.

The advantages of *in vitro* selection are that specific antibody characteristics, such as a slow dissociation rate from the antigen, higher affinity and specificity or even cross specificity, can be selected for, depending on the screening and selection process. The power of *in vitro* screening techniques was demonstrated in two recent studies. Garcia-Rodriguez et al used a yeast display based on co-selection with two *Clostridium botulinum* neurotoxin types to evolve a mAb which originally had a high affinity for toxin type A1 and a low affinity for type A2 to a mAb with a high affinity for both.[19] This proved that it is possible to engineer wider specificity into antibodies by evolving a single antigen binding region to recognise two different epitopes. Another study, which used phage display to increase the affinity of mAbs against the protective antigen (PA) of *Bacillus* (*B.*) *anthracis*, demonstrated that the efficacy of antibodies can be improved by enhancing affinity.[20]

While further *in vitro* manipulation may be required for antibodies generated *in vitro* due to the lower affinity of such antibodies isolated from initial screening, *in vivo* screening enables the isolation of high affinity antibodies that have been affinity matured in the course of selection by the immune system. Methods to immortalise human B cells using the Epstein-Barr virus were developed in the late 1970s[21] and have been used to produce fully human neutralising antibodies against the hepatitis C virus from seropositive donors.[22] In a unique combination of genetic engineering and immunological techniques, transgenic mice carrying human immunoglobulin genes and expressing a fully human repertoire of antibodies have been made, e.g. XenoMouse, and then used to screen for antibodies against a variety of pathogens including SARS coronavirus, HIV-1, *Porphyromonas gingivalis*, *Pseudomonas aeruginosa* and *Cryptococcus neoformans*.[23-27] Another method used to generate mice with human immunoglobulin repertoires is to generate chimeric mice carrying human-derived immune cells. This was done by repopulating mice whose immune system has been eradicated by radiation with functional lymphocytes obtained from human donors (Trimera mice). This system was used to generate XTL001, a combination

of two human mAbs now in clinical trials for prophylaxis of hepatitis B reinfection in liver transplant patients.[28,29]

The success and utility of antibody humanisation and selection techniques can be seen in the increasing sophistication of antibody therapeutics coming on to the market. The first therapeutic antibody approved for use, muromonab (OKT3), in 1986, used to prevent organ rejection, was a murine mAb. Subsequently, chimeric and humanised mAb, basiliximab (Simulect) and daclizumab (Zenapax), both antibodies against IL2, were approved in 1998 and 1997 respectively, for treating the same condition.[7,30] The first cancer therapeutic antibody, rituximab (Rituxan), approved in 1997, was a mouse-human chimera. This was rapidly followed by a humanised antibody produced by CDR grafting, called trastuzumab (Herceptin), and used to treat HER2+ breast cancer, in 1998. The first and only antibody currently approved for the treatment of an infectious disease, palivizumab (Synagis), is a humanised mAb. Recently, adalimumab (Humira), a fully human mAb isolated from a human antibody phage display library, was approved in 2002 and targets TNF-α for the treatment of autoimmune diseases.[31] This wide variety of antibody targets and methods of generation is reflected in their complex nomenclature, described in Fig. 3.

THE EFFICACY OF ANTIBODY THERAPEUTICS IN CURRENT CLINICAL USE

Ultimately, if therapeutic antibodies cannot protect against or treat infection, all techniques for antibody development are irrelevant. The protective effects of antibodies have been known for almost a century. A survey of early 20th century clinical studies on serum therapy for pneumococcal pneumonia and meningococcal meningitis showed a significant improvement in mortality among treated patients.[5] Serum therapy continues today with hyperimmune sera being used for the prophylaxis or treatment of various bacterial and viral diseases, albeit with mixed results. It has been recommended for prophylaxis of viral diseases such as cytomegalovirus (CMV), respiratory syncytial virus (RSV) and hepatitis B as well as for prophylaxis and the treatment of tetanus, botulism and diphtheria, where it functions as an antitoxin.[32] In addition, it is also commonly used as an antivenom for the treatment of stings and snakebites.

A new form of antibody therapy using intravenous immunoglobulin (IVIG) has also been introduced. IVIG is a preparation of human polyclonal antibodies pooled from a large number of healthy donors, and unlike hyperimmune sera, is not enriched for antibodies specific to any particular pathogen. It was initially administered to antibody deficient patients, but has been found to be useful in the treatment of a variety of autoimmune and inflammatory diseases due to its immunoregulatory properties.[33] These properties have also led IVIG to be used for the treatment of sepsis, where it is thought to reduce pathology by suppressing inflammation,[34] although various clinical trials and meta-analyses have reported conflicting results of its efficacy.[35-37] The process by which a successful cure is attained remains unclear and probably relies on processes extrinsic to immune effector mechanisms.

IVIG also appears to provide some benefits in the treatment of certain pathogens. In this regard, it probably functions like hyperimmune sera and relies on immune effector mechanisms such as opsonisation or neutralisation. Commercial IVIG has been shown to neutralise *Staphylococcal* and *Streptococcal* superantigens, opsonise bacteria *in vitro* and protect against infection in mice *in vivo*.[38-40] Positive case studies in human patients have also been reported, but no conclusive clinical trial has yet been conducted.[41,42] In addition, IVIG has been reported to be able to protect against as well as treat complications arising from CMV infection after organ transplantation.[43-45] However, *in vivo* and *in vitro* experimental data, as well as large-scale clinical trials on the efficacy of IVIG, are lacking. Unfortunately, there are also examples for which antibody administration was of limited efficacy, notably in *Mycobacterium tuberculosis* and *Listeria monocytogenes* infection.[46,47] It is noteworthy that many bacterial infections for which antibodies show protective or therapeutic efficacy are those where toxin secretion is a major pathological component, while those infections for which antibodies are less effective are those involving intracellular pathogens. Although antibody-based therapy today still mostly relies on polyclonal preparations, mAbs have also started to make an appearance, with one mAb, palivizumab, approved for prophylaxis against RSV in high-risk neonates, besides many others in the pipeline. Many of these mAbs were developed for infections previously treatable or prevented with polyclonal preparations, such as RSV, hepatitis B, CMV and *S. aureus*. This is not surprising given the inherent advantages of mAbs over polyclonal preparations.

ANTI-BACTERIAL ANTIBODIES

Various studies have shown that targeting bacterial exotoxins is a viable strategy for antibody therapy. *B. anthracis*, the causative agent in anthrax and a potent biological weapon, produces a tripartite exotoxin consisting of a PA, lethal factor (LF) and oedema factor (EF). Post-exposure

prophylaxis with a mAb against PA protected against a lethal inhalational anthrax challenge in rabbits and monkeys, and a mAb against LF protected rats against a challenge with lethal toxin, a combination of PA & LF.[48,49] An anti-PA mAb was also found to act synergistically with the antibiotic ciprofloxacin for protection against inhalational anthrax.[50] Another significant exotoxin-producing pathogen is the Shiga toxin-producing *Escherichia coli*, which causes severe gastrointestinal disease. Complications such as haemolytic uraemic syndrome, acute renal failure and death can result from toxin entry into the bloodstream, and currently, only supportive treatments are available. A human IgG1 mAb generated in transgenic mice against Shiga toxin subunit A prevented fatal systemic complications in piglets following administration after the onset of diarrhoea.[51] Examples of other exotoxins against which mAbs have been shown to have some efficacy include *Pseudomonas aeruginosa* exotoxin A, *Clostridium perfringens* epsilon toxin and *Clostridium botulinum* neurotoxin.[52-54] However, the targeting of exotoxin requires prior knowledge of the pathology of the infectious agent and initial characterisation of the exotoxin.

Of a more generic nature, surface carbohydrates such as lipopolysaccharide (LPS) and lipooligosaccharide contain regions that show relatively little variability between bacteria subtypes and have been explored as potential target antigens. Usually the antibody is targeted against shared or invariant epitopes such as the core carbohydrate backbone, as many bacterial species often exhibit variability in their carbohydrate side-chain residues. In addition, the targeting of LPS may have the advantage of preventing septic shock by promoting the clearance of LPS endotoxin in the bloodstream. However, studies with mAbs against these carbohydrates have shown mixed results. mAbs raised against the inner core LPS of various *Neisseria meningitidis* serotypes have shown poor phagocytic activity despite their avidity for whole cell bacteria and showed poor binding to full-length LPS.[55] Targeting Gram-positive bacteria has shown more positive results. mAbs produced against the deacetylated core carbohydrate backbone of the *S. aureus* surface carbohydrate, poly-N-acetylglucosamine (PNAG), conferred protection from a bacterial challenge in mice and performed better than mAbs against a fully acetylated wild-type PNAG.[56] In another study, mAbs raised against *Streptococcus pneumoniae* serotype 6B capsular polysaccharide with strong cross reactivity for serotype 6A showed avidity-dependent *in vitro* opsonisation and *in vivo* protection against a bacterial challenge with either subtype.[57] Thus, while a generic target for bacteria is attractive, much work is still needed for it to be applied in the clinical setting.

Another area of focus for bacterial antibody therapy is in the treatment of antibiotic-resistant bacteria, such as *S. aureus*. One antibody that has reached the clinical trial stage is tefibazumab (Aurexis), a humanised mAb that binds clumping factor A (ClfA), a major virulence determinant in *S. aureus*. Tefibazumab has been shown to induce phagocytosis of ClfA-coated beads by human polymorphonuclear cells *in vitro*, protect against an intravenous challenge with *S. aureus* in a rabbit model of infective endocarditis and enhance the efficacy of vancomycin therapy in the rabbit therapeutic model.[58] In a phase two clinical trial of tefibazumab in patients with *S. aureus* bacteraemia, the treated group was found to have reduced nasal colonisation, although no significant difference in clinical outcomes was observed between the treated and placebo groups due to the small sample size.[59] Therapeutic antibodies have also been found to be useful in treating fungal infections. Borrowing from the development of antibody-radioisotope conjugates for the treatment of cancer, treatment with an mAb directed against *Cryptococcus neoformans* capsular glucuronoxylomannan labelled with either radioactive bismuth-213 or rhenium-188 improved survival rates of infected mice, although the treatment was not 100% successful.[60]

ANTIBODIES AND VIRAL DISEASE

Palivizumab, the only mAb currently on the market for the treatment of infectious diseases, was developed as a prophylactic treatment against the viral disease RSV. Although mAbs have been shown to be able to neutralise many viral pathogens *in vitro*, the utility of mAb therapy in viral diseases is still a matter of contention as it is unclear to what extent viral clearance depends on antibody-mediated immunity. The clearance of a viral infection is usually associated with T cell-mediated adaptive immunity. CD8+ T cells act by killing virus-infected cells, thus preventing viral replication and reducing the viral load. However, in acute infections, neutralising therapeutic antibodies may still be able to help by suppressing viral replication and viraemia, giving the host immune system time to develop an effective response for viral clearance. In addition, antibodies can promote the killing of infected cells expressing viral proteins on their surface through the activation of natural killer (NK) cells that mediate ADCC, in addition to their viral neutralisation properties.[61,62]

For viral infections where the host immune system is unable to completely clear the virus, leading to a chronic infection, the administration of neutralising antibodies may not be able to achieve complete clearance. In two separate clinical trials using human mAbs against a hepatitis B virus S antigen to treat patients with chronic

hepatitis B infection, the viral DNA and S antigen load in serum were significantly and rapidly reduced after antibody administration.[63,64] Heijtink et al also showed the maintenance of a 90% reduction in S antigen levels 15 days after cessation of therapy in half of the patients, and this correlated with the persistence of the administered mAb in serum. However, in both studies, DNA and S antigen levels eventually recovered once antibody levels in the serum declined following the cessation of therapy. Nonetheless, regular administration of therapeutic antibodies may still prove useful by preventing disease transmission, the infection of healthy cells or the development of pathology through the continued suppression of viral levels.

In other studies with HIV, researchers have shown that the regular administration of therapeutic antibodies may lead to the development of escape mutants. In a landmark clinical trial, a combination of three broadly neutralising HIV antibodies administered over a period of 12 weeks was able to delay viral rebound after the cessation of antiviral treatment as compared to controls. However, viral levels eventually recovered despite the continued administration of all three antibodies. An analysis of viral isolates collected subsequent to antibody administration and during viral rebound found an increasing resistance to the neutralising effect of one of the three antibodies over the course of several months, although the isolates remained sensitive to the other two antibodies.[65] This suggests that the other two antibodies were not able to achieve a neutralising effect *in vivo*, possibly due to low levels in the serum. Thus, the successful treatment of such infections may require continuous administration of a combination of antibodies that cover a broad range of epitopes and at levels that are individually neutralising *in vivo*, reminiscent of current treatments of HIV using multiple drug therapy.

A NICHE FOR ANTIBODY-BASED DRUGS AGAINST EMERGING INFECTIONS?

Despite years of research, certain pathogens, such as HIV, have proved to be extremely difficult to treat with antibody therapy, while other pathogens, such as exotoxin-secreting bacteria, appear to be amenable to treatment. Nevertheless, antibodies have been shown to have prophylactic and therapeutic efficacy against a wide range of viral, bacterial and fungal pathogens. Compared to traditional drugs, the two prime advantages of antibody-based therapeutics are their generally low toxicity and their short-term timescale for development. This has been made possible by the ability of current biotechnology to rapidly generate and manipulate antibodies of various formats with a defined specificity and reduced immunogenicity. Thus, antibodies against a newly discovered pathogen could in theory be generated and brought to clinical use in a relatively short period of time as compared to traditional drugs.

Thus, antibodies are prime candidates for drug development for emerging infections such as SARS and H5N1 avian influenza. Indeed, despite their recent appearance, antibodies have already been made against the above pathogens and tested for their therapeutic efficacy in various animal models. Treatment with a chimeric mouse-human mAb or with mAbs isolated from convalescent human patients was able to completely protect mice infected with the H5N1 virus when administered up to 72 hours post-infection.[66,67] Prophylactic administration of human mAbs against the SARS coronavirus, selected either by phage display or in transgenic mice, was able to reduce the lung viral load in infected ferrets and mice respectively.[68,69] In a study of West Nile virus (WNV) infection in mice, the administration of a high-affinity humanised mAb up to two days after infection afforded complete protection, and administration between three and five days post-infection provided partial protection.[70,71] This protection was partially dependent on Fc function, as mice treated with a mutant mAb defective for complement fixation or FcR binding had reduced survival rates.

However, a point to note on the use of antibody therapeutics is that their efficacy may depend on the immunocompetence of the individual. Antibody treatments that have been successful in protecting immunocompetent animals have been found to be ineffective in treating immunocompromised individuals. For example, the passive administration of human IgG prior and subsequent to infection of WNV in T and B cell-deficient (RAG1 and μMT) mice was unable to completely clear the infection but merely prolonged survival to approximately 35–50 days, in contrast to wild-type mice, which had a 100% survival rate.[72] This is an important consideration if the antibody is to be used to treat pathogens that cause immunodeficiency, e.g. HIV, or infect primarily immunocompromised individuals, e.g. WMV.

CURRENT DRAWBACKS AND FUTURE POSSIBILITIES

The disadvantages of antibody therapeutics are that it is relatively expensive to manufacture, requires systemic administration and is only specific to a particular pathogen or serotype. However, it should be noted that cost estimates for antibodies are based on the first generation of antibodies to hit the market. The widespread use of antibodies is expected to drive the costs down. In addition, much research has been carried out on addressing these disadvantages and recent discoveries have opened up a wealth of options for antibody development (Fig. 4). Advances in protein

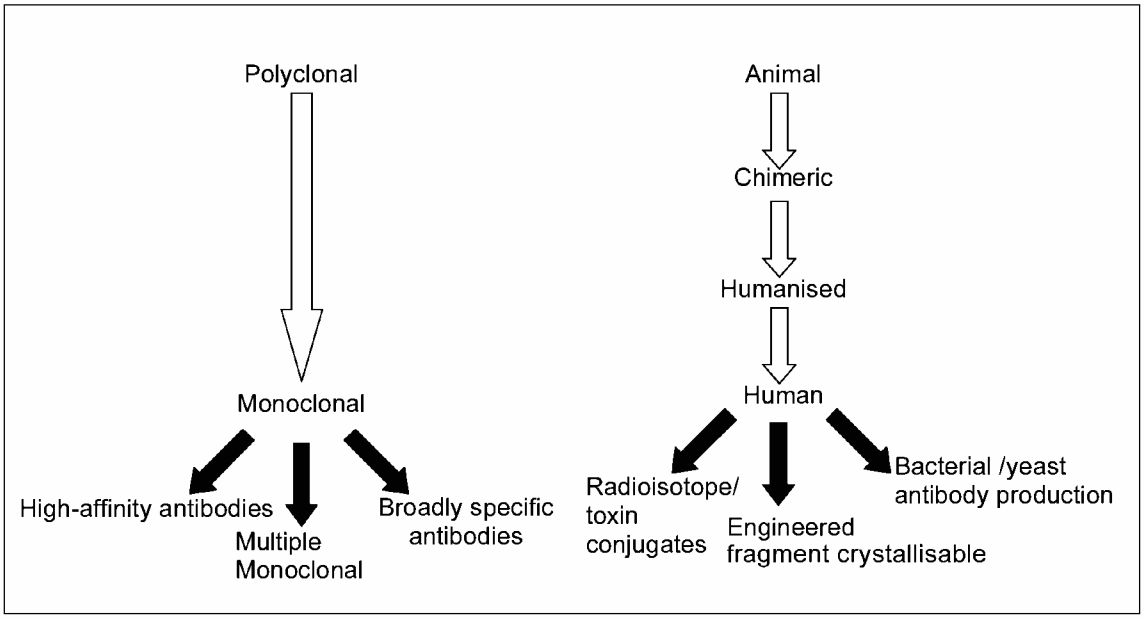

Fig. 4 Diagram shows future directions for antibody technology. Technologies are currently being developed to improve the specificity, effectiveness and to reduce costs. Possible technologies for improving the specificity of antibody-based drugs include formulations of multiple antibodies or selecting antibodies with broad specificity. The efficacy of current unmodified human antibodies can be improved through the use of radioisotope or toxin conjugates, higher affinity antibodies or fragment crystallisable regions engineered to better attract immune system effector functions. Costs of antibody production can also be reduced through production in cheaper expression systems, such as bacteria or yeast.

engineering have made it possible to express antigen-binding fragments (Fab) or even full-length antibodies in bacteria.[73,74] For antibodies requiring glycosylation for efficacy, glycoengineered yeast (*Pichia pastoris*) optimised for expressing recombinant antibodies with human N-glycosylation patterns have been developed.[75] These technologies are expected to bring down both the cost and the time taken to produce antibodies.

Another means to lower costs is by engineering antibodies for higher efficacy so that lower doses are required. One possibility is to engineer antibodies with Fc regions that have a higher affinity for Fc receptors.[76] In doing so, these antibodies would have the advantage in competing with naturally circulating antibodies for the Fc receptors present on immune cells responsible for the effector mechanisms, e.g. NK cells that drive ADCC, and hence require lower levels for equivalent efficacy as compared to unmodified antibodies. Another alternative is to generate higher affinity antibodies, a concept already demonstrated with the high affinity, high efficacy mAbs against *B. anthracis* PA toxin mentioned earlier.[20] The limited potential of a single antibody to target individual serotypes or pathogens can be solved through the use of *in vitro* selection techniques to engineer broadly specific antibodies or by the use of multiple antibody formulations. However, in light of the potential threat of serious emerging diseases such as H5N1 and SARS, even an antibody capable of successfully combating only one such disease would still have great commercial and clinical potential.

With the increasing sophistication of antibody technology and the advantages of antibody-based therapy, we can expect to see an increase in their share of the market for infectious disease therapeutics. Also, even though the majority of currently-approved antibodies were developed to treat cancer and autoimmune diseases, this does not necessarily imply that antibodies are of lesser use in treating infectious diseases, as that is their primary role in the body. Instead, this disparity can be attributed to the priorities of the pharmaceutical and medical communities during that period of development. The percentage of antibody therapeutics in the pipeline today that target infectious diseases is increasing and we shall soon see the effects of this reflected in the drugs approved for the market.

CONCLUSION

Today, modern biotechnology enables researchers to produce fully human antibodies against specific targets using a variety of *in vivo* and *in vitro* screening methods. Concurrently, research is being carried out on improving antibody efficacy, reducing production costs and improving affinity and specificity, with considerable success. With the emergence of new viruses and multidrug resistant bacterial strains, investment in the development of therapeutic antibodies may yield dividends in our clinical preparedness to combat these emerging threats.

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REFERENCES

1. Connolly SE, Thanassi DG, Benach JL. Generation of a complement-independent bactericidal IgM against a relapsing fever Borrelia. J Immunol 2004; 172:1191-7.
2. Moragues MD, Omaetxebarria MJ, Elguezabal N, et al. A monoclonal antibody directed against a Candida albicans cell wall mannoprotein exerts three anti-C. albicans activities. Infect Immun 2003; 71:5273-9.
3. Casadevall A, Pirofski LA. New concepts in antibody-mediated immunity. Infect Immun 2004; 72:6191-6.
4. Winau F, Winau R. Emil von Behring and serum therapy. Microbes Infect 2002; 4:185-8.
5. Casadevall A, Scharff MD. Serum therapy revisited: animal models of infection and development of passive antibody therapy. Antimicrob Agents Chemother 1994; 38:1695-702.
6. 2006 Survey: Medicines in Development: Biotechnology. In: Pharmaceutical Research and Manufacturers Association of America [online]. Available at: www.phrma.org/files/Biotech%202006.pdf. Accessed October 26, 2007.
7. Lin MZ, Teitell MA, Schiller GJ. The evolution of antibodies into versatile tumor-targeting agents. Clin Cancer Res 2005; 11:129-38.
8. Köhler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 1975; 256:495-7.
9. Schroff RW, Foon KA, Beatty SM, Oldham RK, Morgan AC Jr. Human anti-murine immunoglobulin responses in patients receiving monoclonal antibody therapy. Cancer Res 1985; 45:879-85.
10. Dillman RO, Shawler DL, Dillman JB, Royston I. Therapy of chronic lymphocytic leukemia and cutaneous T-cell lymphoma with T101 monoclonal antibody. J Clin Oncol 1984; 2:881-91.
11. Miller RA, Oseroff AR, Stratte PT, Levy R. Monoclonal antibody therapeutic trials in seven patients with T-cell lymphoma. Blood 1983; 62:988-95.
12. Mirick GR, Bradt BM, Denardo SJ, Denardo GL. A review of human anti-globulin antibody (HAGA, HAMA, HACA, HAHA) responses to monoclonal antibodies. Not four letter words. Q J Nucl Med Mol Imaging 2004; 48:251-7.
13. Presta LG. Engineering of therapeutic antibodies to minimize immunogenicity and optimize function. Adv Drug Deliv Rev 2006; 58:640-56.
14. Morrison SL, Johnson MJ, Herzenberg LA, Oi VT. Chimeric human antibody molecules: mouse antigen-binding domains with human constant region domains. Proc Natl Acad Sci U S A 1984; 81:6851-5.
15. Jones PT, Dear PH, Foote J, Neuberger MS, Winter G. Replacing the complementarity-determining regions in a human antibody with those from a mouse. Nature 1986; 321:522-5.
16. Roque-Navarro L, Mateo C, Lombardero J, et al. Humanization of predicted T-cell epitopes reduces the immunogenicity of chimeric antibodies: new evidence supporting a simple method. Hybrid Hybridomics 2003; 22:245-57.
17. Hwang WY, Foote J. Immunogenicity of engineered antibodies. Methods 2005; 36:3-10.
18. Hoogenboom HR. Selecting and screening recombinant antibody libraries. Nat Biotechnol 2005; 23:1105-16.
19. Garcia-Rodriguez C, Levy R, Arndt JW, et al. Molecular evolution of antibody cross-reactivity for two subtypes of type A botulinum neurotoxin. Nat Biotechnol 2007; 25:107-16.
20. Maynard JA, Maassen CB, Leppla SH, et al. Protection against anthrax toxin by recombinant antibody fragments correlates with antigen affinity. Nat Biotechnol 2002; 20:597-601.
21. Tohda H, Oikawa A, Kudo T, Tachibana T. A greatly simplified method of establishing B-lymphoblastoid cell lines. Cancer Res 1978; 38:3560-2.
22. Eren R, Landstein D, Terkieltaub D, et al. Preclinical evaluation of two neutralizing human monoclonal antibodies against hepatitis C virus (HCV): a potential treatment to prevent HCV reinfection in liver transplant patients. J Virol 2006; 80:2654-64.
23. Coughlin M, Lou G, Martinez O, et al. Generation and characterization of human monoclonal neutralizing antibodies with distinct binding and sequence features against SARS coronavirus using XenoMouse. Virology 2007; 361:93-102.
24. He Y, Honnen WJ, Krachmarov CP, et al. Efficient isolation of novel human monoclonal antibodies with neutralizing activity against HIV-1 from transgenic mice expressing human Ig loci. J Immunol 2002; 169:595-605.
25. Lai Z, Kimmel R, Petersen S, et al. Multi-valent human monoclonal antibody preparation against Pseudomonas aeruginosa derived from transgenic mice containing human immunoglobulin loci is protective against fatal pseudomonas sepsis caused by multiple serotypes. Vaccine 2005; 23:3264-71.
26. Maitta RW, Datta K, Lees A, Belouski SS, Pirofski LA.. Immunogenicity and efficacy of Cryptococcus neoformans capsular polysaccharide glucuronoxylomannan peptide mimotope-protein conjugates in human immunoglobulin transgenic mice. Infect Immun 2004; 72:196-208.
27. Shibata Y, Hosogi Y, Hayakawa M, et al. Construction of novel human monoclonal antibodies neutralizing Porphyromonas gingivalis hemagglutination activity using transgenic mice expressing human Ig loci. Vaccine 2005; 23:3850-6.
28. Eren R, Lubin I, Terkieltaub D, et al. Human monoclonal antibodies specific to hepatitis B virus generated in a human/mouse radiation chimera: the Trimera system. Immunology 1998; 93:154-61.
29. Witherell G. XTL-001. XTL Pharmaceuticals. Curr Opin Investig Drugs 2002; 3:684-92.
30. Brekke OH, Sandlie I. Therapeutic antibodies for human diseases at the dawn of the twenty-first century. Nat Rev Drug Discov 2003; 2:52-62.
31. Lawrence, S. Billion dollar babies--biotech drugs as blockbusters. Nat Biotechnol 2007; 25:380-2.
32. Keller MA, Stiehm ER. Passive immunity in prevention and treatment of infectious diseases. Clin Microbiol Rev 2000; 13:602-14.
33. Negi VS, Elluru S, Sibéril S, et al. Intravenous immunoglobulin: an update on the clinical use and mechanisms of action. J Clin Immunol 2007; 27:233-45.
34. Werdan K. Intravenous immunoglobulin for prophylaxis and therapy of sepsis. Curr Opin Crit Care 2001; 7:354-61.
35. Kreymann KG, de Heer G, Nierhaus A, Kluge S. Use of polyclonal immunoglobulins as adjunctive therapy for sepsis or septic shock. Crit Care Med 2007; 35:2677-85.
36. Laupland KB, Kirkpatrick AW, Delaney A. Polyclonal intravenous immunoglobulin for the treatment of severe sepsis and septic shock in critically ill adults: a systematic review and meta-analysis. Crit Care Med 2007; 35:2686-92.
37. Werdan K, Pilz G, Bujdoso O, et al. Score-based immunoglobulin G therapy of patients with sepsis: the SBITS study. Crit Care Med 2007; 35:2693-2701.
38. Sriskandan S, Ferguson M, Elliot V, Faulkner L, Cohen J. Human intravenous immunoglobulin for experimental streptococcal toxic shock: bacterial clearance and modulation of inflammation. J Antimicrob Chemother 2006; 58:117-24.
39. Takei S, Arora YK, Walker SM. Intravenous immunoglobulin contains specific antibodies inhibitory to activation of T cells by staphylococcal toxin superantigens [see comment]. J Clin Invest 1993; 91:602-7.
40. Vernachio JH, Bayer AS, Ames B, et al. Human immunoglobulin

G recognizing fibrinogen-binding surface proteins is protective against both Staphylococcus aureus and Staphylococcus epidermidis infections in vivo. Antimicrob Agents Chemother 2006; 50:511-8.
41. Hampson FG, Hancock SW, Primhak RA. Disseminated sepsis due to a Panton-Valentine leukocidin producing strain of community acquired meticillin resistant Staphylococcus aureus and use of intravenous immunoglobulin therapy. Arch Dis Child 2006; 91:201.
42. Norrby-Teglund A, Muller MP, Mcgeer A, et al. Successful management of severe group A streptococcal soft tissue infections using an aggressive medical regimen including intravenous polyspecific immunoglobulin together with a conservative surgical approach. Scand J Infect Dis 2005; 37:166-72.
43. Bass EB, Powe NR, Goodman SN, et al. Efficacy of immune globulin in preventing complications of bone marrow transplantation: a meta-analysis. Bone Marrow Transplant 1993; 12:273-82.
44. Carbone J, Sarmiento E, Palomo J, et al. The potential impact of substitutive therapy with intravenous immunoglobulin on the outcome of heart transplant recipients with infections. Transplant Proc 2007; 39:2385-8.
45. Weill D, Lock BJ, Wewers DL, et al. Combination prophylaxis with ganciclovir and cytomegalovirus (CMV) immune globulin after lung transplantation: effective CMV prevention following daclizumab induction. Am J Transplant 2003; 3:492-6.
46. Glatman-Freedman A, Casadevall A. Serum therapy for tuberculosis revisited: reappraisal of the role of antibody-mediated immunity against Mycobacterium tuberculosis. Clin Microbiol Rev 1998; 11:514-32.
47. Miki K, Mackaness GB. The passive transfer of acquired resistance to listeria monocytogenes. J Exp Med 1964; 120:93-103.
48. Lim NK, Kim JH, Oh MS, et al. An anthrax lethal factor-neutralizing monoclonal antibody protects rats before and after challenge with anthrax toxin. Infect Immun 2005; 73:6547-51.
49. Vitale L, Blanset D, Lowy I, et al. Prophylaxis and therapy of inhalational anthrax by a novel monoclonal antibody to protective antigen that mimics vaccine-induced immunity. Infect Immun 2006; 74:5840-7.
50. Peterson JW, Comer JE, Noffsinger DM, et al. Human monoclonal anti-protective antigen antibody completely protects rabbits and is synergistic with ciprofloxacin in protecting mice and guinea pigs against inhalation anthrax. Infect Immun 2006; 74:1016-24.
51. Sheoran AS, Chapman-Bonofiglio S, Harvey BR, et al. Human antibody against shiga toxin 2 administered to piglets after the onset of diarrhea due to Escherichia coli O157:H7 prevents fatal systemic complications. Infect Immun 2005; 73:4607-13.
52. Fogle MR, Griswold JA, Oliver JW, Hamood AN. Anti-ETA IgG neutralizes the effects of Pseudomonas aeruginosa exotoxin A. J Surg Res 2002; 106:86-98.
53. McClain MS, Cover TL. Functional analysis of neutralizing antibodies against Clostridium perfringens epsilon-toxin. Infect Immun 2007; 75:1785-93.
54. Smith TJ, Lou J, Geren IN, et al. Sequence variation within botulinum neurotoxin serotypes impacts antibody binding and neutralization. Infect Immun 2005; 73:5450-7.
55. Gidney MA, Plested JS, Lacelle S, et al. Development, characterization, and functional activity of a panel of specific monoclonal antibodies to inner core lipopolysaccharide epitopes in Neisseria meningitidis. Infect Immun 2004; 72:559-69.
56. Kelly-Quintos C, Cavacini LA, Posner MR, Goldmann D, Pier GB. Characterization of the opsonic and protective activity against Staphylococcus aureus of fully human monoclonal antibodies specific for the bacterial surface polysaccharide poly-N-acetylglucosamine. Infect Immun 2006; 74:2742-50.
57. Sun Y, Hwang Y, Nahm MH. Avidity, potency, and cross-reactivity of monoclonal antibodies to pneumococcal capsular polysaccharide serotype 6B. Infect Immun 2001; 69:336-44.
58. Domanski PJ, Patel PR, Bayer AS, et al. Characterization of a humanized monoclonal antibody recognizing clumping factor A expressed by Staphylococcus aureus. Infect Immun 2005; 73:5229-32.
59. Weems JJ Jr, Steinberg JP, Filler S, et al. Phase II, randomized, double-blind, multicenter study comparing the safety and pharmacokinetics of tefibazumab to placebo for treatment of Staphylococcus aureus bacteremia. Antimicrob Agents Chemother 2006; 50:2751-5.
60. Dadachova E, Casadevall A. Treatment of infection with radiolabeled antibodies. Q J Nucl Med Mol Imaging 2006; 50:193-204.
61. Alsmadi O, Tilley SA. Antibody-dependent cellular cytotoxicity directed against cells expressing human immunodeficiency virus type 1 envelope of primary or laboratory-adapted strains by human and chimpanzee monoclonal antibodies of different epitope specificities. J Virol 1998; 72:286-93.
62. Hezareh M, Hessell AJ, Jensen RC, van de Winkel JG, Parren PW. Effector function activities of a panel of mutants of a broadly neutralizing antibody against human immunodeficiency virus type 1. J Virol 2001; 75:12161-8.
63. Galun E, Eren R, Safadi R, et al. Clinical evaluation (phase I) of a combination of two human monoclonal antibodies to HBV: safety and antiviral properties. Hepatology 2002; 35:673-9.
64. Heijtink RA, van Nunen AB, van Bergen P, et al. Administration of a human monoclonal antibody (TUVIRUMAB) to chronic hepatitis B patients pre-treated with lamivudine: monitoring of serum TUVIRUMAB in immune complexes. J Med Virol 2001; 64:427-34.
65. Trkola A, Kuster H, Rusert P, et al. Delay of HIV-1 rebound after cessation of antiretroviral therapy through passive transfer of human neutralizing antibodies. Nat Med 2005; 11:615-22.
66. Hanson BJ, Boon AC, Lim AP, et al. Passive immunoprophylaxis and therapy with humanized monoclonal antibody specific for influenza A H5 hemagglutinin in mice. Respir Res 2006; 7:126.
67. Simmons CP, Bernasconi NL, Suguitan AL, et al. Prophylactic and therapeutic efficacy of human monoclonal antibodies against H5N1 influenza. PLoS Med 2007; 4:e178.
68. 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; 191:507-14.
69. ter Meulen J, Bakker AB, van den Brink EN, et al. Human monoclonal antibody as prophylaxis for SARS coronavirus infection in ferrets. Lancet 2004; 363:2139-41.
70. Morrey JD, Siddharthan V, Olsen AL, et al. Defining limits of treatment with humanized neutralizing monoclonal antibody for West Nile virus neurological infection in a hamster model. Antimicrob Agents Chemother 2007; 51:2396-402.
71. Oliphant T, Engle M, Nybakken GE, et al. Development of a humanized monoclonal antibody with therapeutic potential against West Nile virus. Nat Med 2005; 11:522-30.
72. Engle MJ, Diamond MS. Antibody prophylaxis and therapy against West Nile virus infection in wild-type and immunodeficient mice. J Virol 2003; 77:12941-9.
73. Mazor Y, Van Blarcom T, Mabry R, Iverson BL, Georgiou G. Isolation of engineered, full-length antibodies from libraries expressed in Escherichia coli. Nat Biotechnol 2007; 25:563-5.
74. Simmons LC, Reilly D, Klimowski L, et al. Expression of full-length immunoglobulins in Escherichia coli: rapid and efficient production of aglycosylated antibodies. J Immunol Methods 2002; 263:133-47.
75. Li H, Sethuraman N, Stadheim TA, et al. Optimization of humanized IgGs in glycoengineered Pichia pastoris. Nat Biotechnol 2006; 24:210-5.
76. Shields RL, Lai J, Keck R, et al. Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human Fcgamma RIII and antibody-dependent cellular toxicity. J Biol Chem 2002; 277:26733-40.

SINGAPORE MEDICAL COUNCIL CATEGORY 3B CME PROGRAMME
Multiple Choice Questions (Code SMJ 200907B)

	True	False
Question 1. Regarding the structure and function of antibodies:		
(a) The diversity in the complementarity-determining region enables the immune system to recognise the wide variety of antigens present on pathogens.	☐	☐
(b) The Fc region is not required for the protective functions of the antibody.	☐	☐
(c) Certain protective functions, such as phagocytosis, complement activation and antibody-dependent cellular cytotoxicity, require other components of the immune system.	☐	☐
(d) Antibodies can have direct antibacterial or antifungal activity.	☐	☐
Question 2. Regarding the history of antibody development:		
(a) The first instance of therapeutic use of antibodies was against cancer.	☐	☐
(b) A high incidence of hypersensitivity responses was a factor in the abandonment of serum therapy for antibiotics.	☐	☐
(c) Hybridoma technology is the only method for generating monoclonal antibodies.	☐	☐
(d) Humanised and chimeric monoclonal antibodies have a higher rate of anti-antibody hypersensitivity responses in human patients compared to murine monoclonal antibodies.	☐	☐
Question 3. Regarding the current clinical use of polyclonal antibodies:		
(a) Polyclonal antibody preparations has been recommended for prophylaxis of cytomegalovirus, respiratory syncytial virus and hepatitis B.	☐	☐
(b) IVIG is a type of polyclonal antibody preparation that is not enriched for antibodies against a particular antigen.	☐	☐
(c) Polyclonal antibody preparations have been showed to have good efficacy against intracellular pathogens such as *Mycobacterium tuberculosis* and *Listeria monocytogenes*.	☐	☐
(d) Polyclonal antibody preparations have replaced the monoclonal antibody palivizumab for prophylaxis of respiratory syncytial virus in high-risk neonates.	☐	☐
Question 4. Regarding the development of monoclonal antibodies to treat bacterial infections:		
(a) Post-exposure prophylaxis against anthrax with a monoclonal antibody requires combination therapy with antibiotics to be effective.	☐	☐
(b) Production of antibodies against bacterial toxins requires prior knowledge of the pathology of the infectious agent and initial characterisation of the exotoxin.	☐	☐
(c) Carbohydrates such as lipopolysaccharide and lipooligosaccharide on bacterial surfaces have been explored as targets for therapeutic antibodies.	☐	☐
(d) Targeting the core carbohydrate backbone is preferred because many bacterial species often exhibit variability in their carbohydrate side-chain residues.	☐	☐
Question 5. Regarding the use of therapeutic antibodies in viral infection:		
(a) Recognition of viral proteins by antibodies enables natural killer cells to take up and kill individual viral particles.	☐	☐
(b) Administration of monoclonal antibodies against hepatitis B S antigen in clinical trials successfully suppressed viral load and cleared hepatitis B infection.	☐	☐
(c) The failure of antibody therapy to clear HIV infection is partially due to the development of escape mutants.	☐	☐
(d) Antibodies can be rapidly generated against emerging viral infections, such as SARS and H5N1 avian influenza.	☐	☐

Doctor's particulars:

Name in full: _____

MCR number: _____ Specialty: _____

Email address: _____

SUBMISSION INSTRUCTIONS:
(1) Log on at the SMJ website: http://www.sma.org.sg/cme/smj and select the appropriate set of questions. (2) Select your answers and provide your name, email address and MCR number. Click on "Submit answers" to submit.

RESULTS:
(1) Answers will be published in the SMJ August 2009 issue. (2) The MCR numbers of successful candidates will be posted online at www.sma.org.sg/cme/smj by 15 September 2009. (3) All online submissions will receive an automatic email acknowledgment. (4) Passing mark is 60%. No mark will be deducted for incorrect answers. (5) The SMJ editorial office will submit the list of successful candidates to the Singapore Medical Council.

Deadline for submission: (June 2009 SMJ 3B CME programme): 12 noon, 7 September 2009.