

## Opportunities and Challenges of Therapeutic Monoclonal Antibodies as Medical Countermeasures for Biodefense

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### Abstract

Antibodies, naturally produced in the body as part of the immune response to infectious agents, can also be introduced artificially to treat infectious diseases. Advances in biotechnology in the last decades have made human or humanized monoclonal antibodies (mAbs) as therapeutics possible. These therapeutic mAbs currently enjoy unprecedented success and recognition of their potential. Unlike vaccines, therapeutic mAbs can confer instant and consistent protection against bio-threat agents when administered regardless of the recipient's immune status. Therapeutic mAbs can be administered in higher levels than those elicited by vaccines, and thus provide a higher level of protection or treatment that is necessary in a biological attack where people are exposed to a higher exposure of agent concentration than that found in nature. Furthermore, therapeutic mAbs have substantial advantages over antimicrobial drugs, such as high specificity, low systemic toxicity, relatively long half-life, and no concerns over disrupting the body's microbiome. Therapeutic mAbs can be used for both pre- and post-exposure protection; therefore, they have great value as effective medical countermeasures (MedCMs) against bio-threat agents. However, there are still some challenges to be overcome before therapeutic mAbs become ideal MedCMs against bio-threat agents. In this review, both opportunities and challenges in development of therapeutic mAbs are discussed.

**Keywords:** Therapeutic; Monoclonal antibodies; Biodefense

### Introduction

A bio-threat agent is a biological pathogen or toxin that can be used purposefully as a weapon in bioterrorism to spread life-threatening diseases on a large scale in order to cause fear, disorganization, and panic in the public. Numerous potentially weaponizable bio-threat agents have been described and studied. It is impossible to predict when and where, a bioterrorist attack is going to happen, let alone predict which agent would be used.

Vaccination can induce protective immune responses and then protect the vaccinated population against specific bio-threat attacks. Unfortunately, vaccines require time (weeks or months) to take effect. The required time period is usually longer than that between bio-threat exposure and onset of diseases. Moreover, vaccination does not guarantee that all the vaccine recipients would mount protective responses. It is estimated that around 10% of recipients do not mount any protective responses after vaccination [1]. These drawbacks of vaccines would limit their usefulness during an emergency response to a potential bio-threat scenario. Besides, it is also controversial to vaccinate a whole population or large number of individuals for pre-exposure prophylaxis against an uncertain and unpredictable biological attack [2].

Antimicrobial drugs are very effective against various bacteria, but obviously ineffective at eliminating viral infections. Antimicrobial drugs can provide protection when administered after exposure, however they have a short serum half-life (hours) and resistance often emerges after repeated use [3]. More than 70% of hospital-acquired bacterial infections are resistant to at least one of the drugs typically

used to combat them [4]. Resistance to multiple drugs is increasing. In addition, most antibiotics are broad-spectral, killing commensal bacteria and leading to imbalanced gut micro flora in the body. Furthermore, there is also a concern regarding the intentional bioengineering of anti-microbial resistance in potential bio-threat agents. While clearly there is a critical need for new antibiotics and antiviral drugs not only for biodefense, but also to combat naturally occurring infectious diseases, unfortunately, new therapeutics are years from being developed and approved [5].

Antibodies, naturally produced in the body, are highly versatile immune-defense molecules to counteract pathogens (bacteria, viruses, toxins, etc.). As a matter of fact, antibodies have been used as MedCMs against infectious diseases since the late 19th century [6,7].

### Animal anti-serum

Antibody therapy against infectious diseases was discovered by the German physiologist, Emil Adolf von Behring a century ago [7,8]. His work led to the first instance of industrial production of protective serum from horses in 1893 and to his receipt in 1901 of the first Nobel Prize in medicine. Following Behring's discovery, animal immune serum (anti-serum) was used to treat bacterial, viral, and toxin-mediated diseases where a protective immune response could be induced in the animal host by vaccination. However, the use of animal anti-serum was not entirely safe. Due to its foreignness to humans, its use gave rise to anaphylaxis (serum sickness). Therefore, the use of animal anti-serum was largely abandoned by the 1930s.

## Human anti-serum

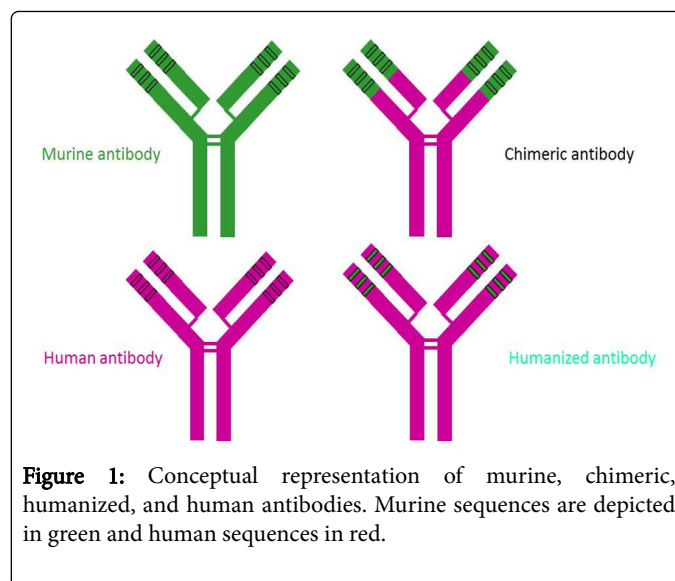
Replacing animal anti-serum, human anti-serum from convalescent or immunized subjects came into use. Despite unquestioned efficacy in clinics, human anti-serum therapy suffered from a number of drawbacks, including high cost, limited source, cumbersome, batch-to-batch variation, low content of specific antibodies (only 1% of the total antibodies), uncertain dosing, and a risk of infection from blood borne products. Subsequently, human anti-serum therapy against bacterial infections was largely abandoned in the 1940s when antibiotics came into clinical practice. However, human anti-serum therapy for viral and toxin-mediated diseases for which there are no alternative therapeutic options has continued to develop. Recently, the serum in anti-serum therapy has been replaced by serum-derived immunoglobulin (polyclonal antibodies) from pooled human donors. The risk from adventitious agents has been reduced by better screening of donors and new production methods which inactivate or remove viruses. These hyper immune human serum-derived immunoglobulin products approved by the U.S. Food and Drug Administration (FDA), are mainly used for viruses (such as cytomegalovirus, respiratory syncytial virus, hepatitis A virus, hepatitis B virus, rabies virus, vaccinia virus, varicella-zoster, and measles virus) underscoring the fact that antibody therapy remains an effective means of treatment against viruses [9-12].

## Monoclonal antibodies (mAbs)

In 1975, the development of mAbs by murine hybridoma technology opened a new era in antibody therapy. In contrast to polyclonal antibodies (serum-derived immunoglobulin), mAbs are derived from a single cell line and are thus identical in binding sites and binding affinities [13]. Using this technology, it has been possible to produce large amounts of antibodies of the desired specificity. At the time, it was widely believed that mAbs would be the “magic bullets” for therapy. However, the early excitement was rapidly replaced by disappointment when it was found that mAbs, like animal anti-sera, had serious side effects in humans mainly due to its “foreignness” to humans [14], such as rapid clearance of mAbs from humans and potentially dangerous anaphylactic response. Furthermore, the mouse antibody constant region does not interact properly with the human immune system and results in inefficiency at exerting therapeutic effects [15]. A tremendous effort was devoted to make human mAbs. However, it was soon found that hybridoma technology was unable to produce human mAbs.

## Chimeric, humanized, and human mAbs

As molecular biology technology advanced in the mid-1980s, two approaches were developed to minimize the potential of non-human sequence for unwanted and potentially dangerous immune responses in humans and enhancement of therapeutic effects of well-characterized murine mAbs. This was accomplished by replacing some parts of the murine antibody with human antibody counterparts: chimerization and humanization as shown in (Figure 1) [16-22].



**Figure 1:** Conceptual representation of murine, chimeric, humanized, and human antibodies. Murine sequences are depicted in green and human sequences in red.

In murine/human chimeric antibodies, the murine constant region (around 70% of total antibody sequence) is replaced by the human counterpart. Chimeric antibodies successfully retain the original murine antibody variable region (antigen-binding specificity) and possess a fully human antibody constant region, which make them considerably less immunogenic in humans and allows interaction with the human immune system to exert indirect effects. However, chimeric antibodies may still elicit an undesirable response to the murine antibody variable region [23].

In order to further reduce chimeric antibody immunogenicity, humanization was developed in 1988 to replace the murine antibody frameworks with those of selected human antibodies on chimeric antibodies [19]. The resulting “humanized” antibodies contained 90-95% human sequences [24-27]. Numerous clinical studies have confirmed that humanized antibodies are less immunogenic and more therapeutic in humans than murine or chimeric antibodies [28,29]. However, the process of humanization of murine antibodies is much more challenging than construction of murine-human chimeric antibodies. Humanization may result in a loss of antibody antigen-binding activity. Nevertheless, humanization has played a fundamental role in the remarkable progress of antibodies as therapeutics.

An alternative to humanization is the derivation of fully human antibodies directly from *in vitro* phage display libraries of human antibodies, in which human antibody fragments are displayed on the surface of phages. Unlike the antibodies developed *in vivo*, these antibodies possess only limited antigen-binding affinity due to the absence of assistance from the *in vivo* immune system for affinity maturation by somatic hyper-mutation. Another approach has been to use transgenic mice modified to carry human antibody genes [30-32]. Immunization of these mice leads to the development of human antibodies, from which hybridomas that produce human antibodies can be generated. However, these mice cannot be used effectively when the targeted antigen is either toxic to mice or homologous with murine tissues. Because of these reasons, the majority of therapeutic mAbs approved by the FDA and in clinical trials are humanized mAbs. Therefore, humanization remains an attractive and proven strategy for switching well-characterized and highly specific murine antibodies into clinical therapeutics.

The technology breakthrough for making humanized or fully human mAbs has lessened the barrier for mAbs as therapeutics. Consequently, mAb therapy has had unprecedented success, growth in research and revenues, and recognition of its potential. This success is likely due to human/humanized mAbs being much better than human hyper immune immunoglobulin as therapeutics. It is undoubtedly revolutionizing the practice of medicine and truly realizing the earlier vision of “magic bullet” medicine. Since 2000, the therapeutic market for mAbs has grown rapidly and therapeutic mAbs have become the fastest growing class of therapeutic agents. Currently nearly all large pharmaceutical companies have at least one mAb licensed product and more candidates in their pipelines. As of 2014, there were around 50 therapeutic mAbs on the market and 300 more in clinical trials [33].

### Highlights of human or humanized therapeutic mAbs as MedCMs for biodefense

**Instant and consistent protection:** Able to confer immediate protection to recipients regardless of recipient's current immune status.

**Higher than natural protection:** Can be administered in higher level and then provide greater protective immunity than vaccination.

**High specificity:** Can be developed only to target specific components of bio-threat agents without risk of cross-reactivity with human tissue.

**Low toxicity:** Much more acceptable and tolerable than other types of MedCMs in humans due to their natural components within the body.

**Reasonable duration:** Because of the plasma half-life of 20 days, the protective immunity provided by passive mAbs can last for weeks after a single administration. It can be extended with additional administrations of mAbs, but it will not last for a lifetime of the recipient.

**Double effects:** Able to neutralize bio-threat agents by themselves and interact with host immune system to destroy bio-threat agents.

**Short development time to licensure:** Can be developed against most bio-threat agents, if not all within a shorter period of time than other types of MedCMs.

**High regulatory approval rate:** Higher approval rate than that of other drug classes due to the well-defined and understood pathway for licensure approval.

**Scientific evidence on mAb effectiveness against many bio-threat agents** [34-38].

**Uniqueness in neutralizing toxins:** Currently, no drugs are available that specifically counteract toxin-mediated intoxication, while toxin neutralization is a classical property of antibody-mediated protection.

### Challenges

There are still certain challenges remaining for the human or humanized therapeutic mAbs as ideal MedCMs for biodefense.

#### Manufacturing cost

Therapeutic mAbs are among the most expensive drugs. The current production of therapeutic mAbs requires the use of very large cultures of mammalian cells followed by extensive purification steps, under Good Manufacturing Practice (GMP) conditions, leading to extremely

high production costs (\$300/gram) [39]. As a result, the FDA-approved anti-anthrax recombinant human antibody, Raxbacumab cost \$5,100 for each dose when stockpiled. The high cost has adversely affected the development of mAbs as therapeutics.

The cost might be reduced by certain new technologies, such as plant-based mAb production. Plants can be used as bioreactors for large scale production of therapeutic antibodies at a low cost [40,41]. Significant progress has been made in the expression of mAbs in plants. In the first human trial of a mAb produced in transgenic tobacco plants, the mAb was demonstrated to provide protection against oral *Streptococcus mutans* colonization [42]. Preclinical studies of a humanized anti-herpes simplex virus (HSV) mAb produced in transgenic soybean plants showed that the mAb prevented transmission of HSV infection in mice [43]. Recently, the humanized anti-ricin therapeutic antibodies and anti-Venezuelan equine encephalitis virus (VEEV) therapeutic antibody were produced in plants at Defence Research and Development Canada, Suffield Research Centre. These two plants produced therapeutic antibodies have been confirmed to be comparable to their mammalian cell-produced counterparts in terms of anti-ricin and anti VEEV infection potency (publication in preparation). These results suggest promise for the use of this technology.

Plant systems have several advantages over mammalian cell culture. They are fast, efficient, highly versatile (for new product development), and easily scalable with significantly reduced manufacturing costs. In addition, they are free from contamination by mammalian pathogens. However, before plant-produced therapeutic mAbs come to the market, the plant production system for therapeutic mAbs has to meet the rigorous regulatory requirements and standards for pharmaceutical products.

#### Antibody efficacy

Antibody therapies are usually administered intravenously due to the large amount of antibody required. For example, the treatment regimen of raxibacumab (humanized mAb against inhalational anthrax, recently approved by the FDA) is 40 mg/kg [44]. Such an amount of mAb has to be administered by intravenous infusion, which takes at least 2 hours. Infusions on the order of hours require a specialized capability in a hospital environment. This approach would be impractical when large populations are exposed to a biological attack. To be effective at a large population-scale, delivery should be intramuscular (less volume), which requires higher potency of mAbs in the formulation than is currently available. The small amount of mAbs with higher efficacy could be supplied in self-injectable devices that allow victims to protect themselves upon notification of bio-threat attack. Two aspects should be taken into account in order to improve antibody efficacy:

**Direct antibody effects:** The antigen target (where the antibody binds) is crucial for the antibody to exert its neutralizing function. If the antibody binds to the pathogen or toxin at the right location, the antibody will efficiently block the entry of pathogen or toxin into cells; otherwise, the blockage will only be partial or null. For example, a recently developed anti-ricin mAb showed extremely high efficacy since it binds to the part of ricin which is responsible for binding to the cell surface to initiate ricin entry into cells [45]. Only a small amount of antibody (0.25 mg/kg) was required for complete protection against lethal ricin challenge [26,45-47].

**Indirect antibody effects:** The antibody constant region is responsible for interacting with immune system to recruit immune components to destroy the target cells. Engineering of constant region has demonstrated enhanced constant-region functionality (indirect effects) by 100 folds [48].

### Cocktail of mAbs

Although specificity is the strength of mAbs, a bio-threat agent that may undergo rapid mutation to result in antigenic variation poses a significant hurdle for mAbs as therapeutics. For example, the high mutation rate of certain viruses enables them to escape neutralization [49]. This problem may be overcome by using mixtures of mAbs that target various areas of bio-threat agents. Several studies demonstrated that combination therapy with mAb cocktails could prevent escape variants for many viruses including influenza, coronavirus, and lymphocytic choriomeningitis virus [50-52]. Also, a cocktail of functional mAbs could provide more protection and target more microbial strains than a single one. The inclusion of multiple mAbs in any therapeutic formulation could be fraught with complex regulatory and licensing issues. However, just recently the FDA has allowed cocktails of mAbs to be clinically tested as one product due to the administration of a cocktail of mAbs required for many infectious disease indications [53].

### Broad spectrum

Currently developed or developing anti-infective mAbs are only specific to one pathogen or toxin per antibody product. The new generation of therapeutic mAbs could be developed against multiple pathogens or toxins by a single mAb product. For this scenario to be realistic, there are two kinds of strategies:

**Bio-threat agent-targeted mAbs:** MAbs should be against certain conserved regions shared within a group of bio-threat agents. For example, antibodies may be developed against novel targets, such as those that regulate microbial growth and virulence factor expression, or are used by a subset of microbes to infect host cells [54,55]. To fight multiple drug resistance bacteria, some successful approaches have targeted the cellular efflux pumps responsible for antibiotic resistance [56]. These types of "broad-spectrum" mAbs are ideal therapeutics for MedCMs against unpredictable and uncertain future biological threats.

**Host-targeted mAbs:** Over the past years therapeutic antibodies have been developed to target pathogen or toxin components. The common cellular pathways used by a wide range of pathogens or toxins have been missed as therapeutic targets. Our understanding of host-pathogen or toxin cellular interactions involved in bio-threat agent pathogenesis remains minimal. The research on this field needs to be emphasized to develop the knowledge for identifying host targets and then host-targeted antibodies could be developed as broad-spectrum therapeutics. These host-targeted mAbs should be in a Fab or F(ab')<sub>2</sub> format in order to eliminate potential adverse effects of antibody-dependent cellular or complement cytotoxicity via the Fc portion.

### Pharmaceutical properties

**Long-lasting:** Despite the reasonably long half-life of therapeutic antibodies (around 20 days), for MedCMs against bio-threat agents, a long serum half-life is much desirable as it would decrease the need for repeat injections of mAbs to achieve a therapeutically relevant serum concentration. Recent studies showed that modification in the constant

region of antibodies could extend up to four-fold their half-life while retaining efficacy [57,58].

An alternative approach is to take advantage of the body's natural ability to express transgenes to produce passive antibodies. This approach can be achieved by *in vivo* delivery of genes encoding bio-threat agent-specific antibodies for biodefense applications [59]. Animal studies have shown that the expressed antibodies can be detected as early as day 3, reach peak levels at day 7, and maintain therapeutic levels in serum for more than seven months after a single administration via antibody gene delivery [60]. Therefore, antibody gene delivery *in vivo* might be a new approach for post-exposure prophylaxis or therapy and for pre-exposure prophylaxis of bio-threat agent-mediated diseases although there are still some problems to be overcome before this new approach could actually be used in humans.

Stem cells can be used as an antibody gene delivery platform to provide relatively long passive immunity against a pathogen. In a previous study, a single intramuscular injection of anti-VEEV-engineered stem cells was demonstrated to generate and maintain higher circulating anti-VEEV antibody titers. Most critically, pre-treatment with engineered stem cells significantly improved both survival and morbidity after exposure to a high lethal dose of highly virulent VEEV. Engineered stem cells maintained the protective anti-VEEV titers for up to 38 days after implantation into mice, and continued to secrete anti-VEEV antibody for over months [61]. Thus, engineered stem cells represent an attractive antibody delivery modality that improves the efficacy of already existing prophylactic countermeasures to infectious disease.

A single dose of antibody administration for post-exposure treatment is like one stone to kill two birds, that is, not only to provide immediate protection to the victim against infections/intoxications, but also quickly elicit a host immunity against infections/intoxications that subsequently results in long term protection (5 months). In a murine ricin intoxication model, one dose of anti-ricin antibody administration 1 hour after 5 × LD<sub>50</sub> ricin challenge rescued the mice. Nine days later, when the rescued mice received a second ricin challenge (5 × LD<sub>50</sub>), the mice still survived [62]. The experimental design excluded the possibility of residual passive antibody responsible for the protection against the second ricin challenge. Results confirmed that the active immunity against ricin in mice was induced quickly following the passive delivery of a single dose of anti-ricin antibody post-exposure. The mechanism for this phenomenon is that the ricin bound by the passive antibody can be captured and internalized efficiently by antigen-processing cells (APC) through the binding of passive antibody Fc portion to the Fc receptor on the APC and thereby dramatically elicit active anti-ricin immunity immediately.

### Increasing stability

Another complication with antibodies is that they usually need to be kept cold to be stable. This is inconvenient for deploying an antibody-based strategy for biodefense or to any field operation. Technology advances in antibody engineering could allow development of stable preparations with their storage at room temperature as solutions. In a recent report, when some mutations were introduced, the antibody stability was improved dramatically [63].

### Conclusions

Therapeutic mAbs have great value as effective MedCMs against bio-threat agents. Although there are still some challenges to be

overcome before therapeutic mAbs become ideal MedCMs against bio-threat agents. With continuous advances in antibody technology and in the understanding of infectivity and intoxication of bio-threat agents, a new generation of therapeutic mAbs with extraordinary efficacy could be developed and these therapeutic mAbs would be not only fast-acting, but also cost-effective and long-lasting against unpredictable and uncertain future bio-threat agents for biodefense.

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