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Development of antibodies as medical countermeasures to biothreat agents

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Abstract

Antibodies have great potential for countering biothreat agents that are not addressed by current medical countermeasures. They are highly versatile defence molecules naturally produced in the body and can be developed against any biothreat agent. Antibodies can directly neutralize biothreat agents and/or invoke destruction of them through the assistance of the immune system. Unlike vaccines, which require time to induce protective immunity and depend on the host's ability to mount immune responses, therapeutic antibodies can confer instant and consistent immunity against biothreat agents once administered regardless of the immune status of the host. In addition, therapeutic antibodies have substantial advantages over antimicrobial drugs, such as high specificity, low toxicity, and long serum half-life (around 20 days). Therapeutic antibodies can be used, either prophylactically to prevent diseases before, or therapeutically to treat diseases after biothreat attacks. It is these properties that make antibodies attractive for medical countermeasures against biothreat agents. As reviewed here, the development of therapeutic antibodies against biothreat agents is summarized in the following areas: history of using antibodies against infectious diseases, current status of development of therapeutic antibodies against an extensive list of biothreat agents, and a table summarizing the development stages (discovery, pre-clinical, and clinical) of each anti-biothreat agent antibody.

Résumé

Les anticorps sont des outils très prometteurs pour contrer les agents de menace biologique non neutralisés par les contre-mesures médicales actuelles. Ce sont des molécules de défense très polyvalentes naturellement fabriquées par l'organisme et qu'on peut mettre au point contre tout agent de menace biologique. Les anticorps peuvent directement neutraliser les agents de menace biologique ou amener leur destruction par le système immunitaire. Contrairement aux vaccins, qui nécessitent un délai pour induire une immunité protectrice et qui dépendent de la capacité de l'hôte à bâtir une réponse immunitaire, une fois administrés, les anticorps thérapeutiques confèrent une immunité instantanée et constante contre un agent de menace biologique, quel que soit l'état immunitaire de l'hôte. En outre, les anticorps thérapeutiques présentent des avantages appréciables par rapport aux antimicrobiens, par exemple une grande spécificité, une faible toxicité et une longue demi-vie sérique (environ 20 jours). Les anticorps thérapeutiques peuvent être utilisés dans un but prophylactique, pour prévenir les maladies, ou dans un but thérapeutique, pour traiter les maladies après une attaque par un agent biologique. Ce sont ces propriétés qui rendent les anticorps si intéressants comme contre-mesures médicales en présence d'agents de menace biologique. Dans le présent document, divers aspects de la mise au point d'anticorps thérapeutiques contre les agents de menace biologique sont résumés : historique du recours aux anticorps pour lutter contre les maladies infectieuses, état actuel de la mise au point d'anticorps thérapeutiques dirigés contre de nombreux agents de menace biologique et tableau résumant les étapes de la mise au point (découverte, essai préclinique et essai clinique) de chacun des anticorps dirigés contre un agent de menace biologique.

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Executive summary

Development of Antibodies as Medical Countermeasures to Biothreat Agents

Wei-Gang Hu; DRDC Suffield TM 2012-120; Defence R&D Canada – Suffield; December 2012.

Introduction: Current medical countermeasures against biothreat agents consist primarily of vaccines and antimicrobial drugs. Both suffer from certain limitations. Vaccinations take a number of weeks and possibly several administrations of vaccine before they are effective, while resistance to antibiotics is becoming increasingly widespread and only a limited number of antiviral drugs are available. Antibodies — proteins which are naturally produced in the body as part of the immune response to infectious agents, many noxious substances, and vaccines — can also be introduced artificially to treat diseases, provided an external source of antibodies is available. Antibodies from animals were first employed to treat disease over a century ago, but their use was always limited, as they themselves produce an adverse immune response in humans. However, through advances in biotechnology, it is now possible to produce human or humanized antibodies on a large scale *in vitro* for use as therapeutics.

Results: This technical memorandum reviews the state of development of therapeutic antibodies against a wide range of biothreat agents, including emerging diseases, that the Canadian Armed Forces (CAF) may potentially encounter during operations. In the case of anthrax (*Bacillus anthracis*, agent N), effective therapeutic antibodies have already entered production and others are in advanced stages of development. While little work has yet been conducted some threat agents, significant advances have been achieved against many others. However, against some threat agents, notably intracellular parasites such as *Burkholderia pseudomallei* (agent HI), the causative agent of melioidosis, candidate therapeutic antibodies have shown meagre efficacy and effective antibodies might never be developed.

The research and development program at DRDC Suffield in this area compares favourably with that underway elsewhere. DRDC Suffield has established a series of novel approaches to developing therapeutic antibodies against biothreat agents, which have resulted in a number of therapeutic antibodies developed to Technology Readiness Levels 3 or 4. Its recent humanized anti-ricin antibody is the most efficacious ever developed.

Significance: Therapeutic antibodies offer the potential for instant, consistent, and high-level protection against a biothreat agents and emerging diseases, and could afford significant protection to Canadian Armed Forces personnel, especially those engaging in rapid deployment missions.

Future plans: DRDC Suffield's efforts in this area are focused on developing its humanized anti-ricin antibody to the preclinical stage, in a project supported by the Canadian Forces Health Services. The United States National Institutes of Health is also considering providing NIH-funded services for the further development of this antibody.

Sommaire

Development of Antibodies as Medical Countermeasures to Biothreat Agents

Wei-Gang Hu ; DRDC Suffield TM 2012-120 ; R & D pour la défense Canada – Suffield; décembre 2012.

Introduction ou contexte : Les contre-mesures médicales utilisées actuellement contre les agents de menace biologique comprennent principalement des vaccins et des antimicrobiens. Les vaccins et les antimicrobiens présentent toutefois certaines limites. Les vaccins nécessitent plusieurs semaines et possiblement plusieurs administrations avant d'être efficaces. Par ailleurs, la résistance aux antibiotiques est de plus en plus répandue, et les antiviraux sont peu nombreux. Des anticorps — protéines fabriquées naturellement par l'organisme dans le cadre d'une réponse immunitaire contre des agents infectieux, de nombreuses substances toxiques et des vaccins — peuvent aussi être introduits dans l'organisme de façon artificielle pour traiter des maladies, si une source externe est existante. Les anticorps d'animaux ont d'abord été employés pour traiter les maladies il y a un siècle, mais leur utilisation a toujours été limitée, car ils stimulent eux-mêmes une réponse immunitaire indésirable chez l'humain. Cependant, grâce aux progrès de la biotechnologie, il est maintenant possible de produire des anticorps humains ou humanisés à grande échelle in vitro à des fins thérapeutiques.

Résultats : Dans le présent document technique, les auteurs examinent l'état d'avancement des travaux de mise au point d'anticorps thérapeutiques dirigés contre une vaste gamme d'agents de menace biologique, notamment des agents causant des maladies émergentes, avec lesquels les Forces armées canadiennes (FAC) pourraient entrer en contact pendant les opérations. Dans le cas du charbon (*Bacillus anthracis*, agent N), des anticorps thérapeutiques efficaces sont déjà en cours de production, et la mise au point d'autres anticorps est à un stade avancé. Jusqu'à maintenant, peu de travaux ont été menés sur certains agents de menace, mais des progrès importants ont été réalisés pour de nombreux autres. Toutefois, dans le cas de certains agents de menace, notamment les parasites intracellulaires comme *Burkholderia pseudomallei* (agent HI), l'agent responsable de mélioïdose, il est peut-être impossible de mettre au point des anticorps efficaces étant donné que les anticorps thérapeutiques candidats se sont révélés peu efficaces.

Le programme de recherche et de développement de RDDC Suffield en ce domaine se compare favorablement aux programmes en cours ailleurs. RDDC Suffield a établi plusieurs nouvelles méthodes de mise au point d'anticorps thérapeutiques dirigés contre des agents de menace biologique, qui ont donné plusieurs anticorps thérapeutiques dont le stade de développement technologique est de 3 ou de 4. L'anticorps anti-ricine humanisé récemment mis au point est le plus efficace jamais créé.

Importance : Les anticorps thérapeutiques offrent la possibilité d'une protection instantanée, durable et d'envergure contre des agents de menace biologique et des agents causant des maladies émergentes, et pourraient conférer une bonne protection aux militaires des Forces armées canadiennes, en particulier ceux qui doivent partir rapidement en mission.

Perspectives : Les efforts déployés par RDDC Suffield dans ce domaine sont axés sur la mise au point d'un anticorps anti-ricine humanisé jusqu'au stade préclinique, dans le cadre d'un projet financé par les Services de santé des Forces canadiennes. Les National Institutes of Health des États-Unis envisagent aussi de fournir des services financés par eux et destinés aux futures étapes de la mise au point de cet anticorps.

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1 Introduction

In the course of their duties, Canadian Armed Forces (CAF) personnel are potentially at risk of being deliberately exposed to biological warfare/bioterrorism agents and are also at risk of exposure to endemic diseases in areas to which they are deployed, as well as to emerging pathogens. These biological hazards can be collectively considered to be "biothreat agents". Possible medical countermeasures against biothreat agents mainly include vaccines, antimicrobial drugs, and antibodies.

Vaccination can induce protective immune responses and then reduce the susceptibility of a population against specific biothreat agents. Unfortunately, vaccines require time (weeks) to induce protective responses. The required time is usually longer than the one between exposure and onset of infectious diseases. Moreover, many vaccines require multiple doses over a time course of weeks or even months to achieve protective responses. Meanwhile, vaccination does not guarantee that all vaccine recipients would mount protective responses. For example, around 10% of individuals receiving vaccine against hepatitis B virus do not elicit any protective responses [1]. These drawbacks of vaccines would limit their usefulness during an emergency response to a potential biothreat situation. Therefore, vaccination is not appropriate for post-exposure medical countermeasures against biothreat agents. Meanwhile, it is also controversial to vaccinate a whole population or large number of individuals for pre-exposure prophylaxis against an uncertain and unpredictable biothreat attack unless there is a clear and present threat.

Antimicrobial drugs are effective to kill bacteria, but ineffective to eliminate viral infections. Although antimicrobial drugs can provide protection when administered after exposure, their serum half-lives are short (hours) and the resistance often occurs after repeated use [2]. More than 70% of the bacteria causing hospital-acquired infections are resistant to at least one of the drugs specially used to combat them. Resistance to multiple drugs is increasing. Clearly there is a critical need for new antimicrobial drugs not only for biodefense, but also for treatment of naturally occurring infectious diseases.

Antibodies, which are glycosylated proteins, naturally produced in the body, have a high specificity and affinity to foreign substances, playing an important role in the immune defence. Antibodies are far more versatile natural reagents than once thought. Antibody functions include two principal actions. The first is direct effects [3], which appear to be a function of antibody antigen-binding fragment (Fab) for toxin neutralization, viral neutralization, and interference with microbial attachment or replications. The second is indirect effects also called effector functions [4], which result from the interaction of antibody constant region, also called crystallisable fragment (Fc) with immune components. Through Fc, antibodies can recruit either the complement cascade, leading to the formation of pores in the targeted cell membrane to exert complement-defendant cytotoxicity (CDC), or effector cells to destroy the target cells to induce antibody-dependent cellular toxicity (ADCC).

Unlike vaccines, therapeutic antibodies can provide immediate immunity to all recipients when administered regardless of recipient's ability to mount immune responses. Antibodies may be administered in high levels, which exceed that elicited by vaccines, and thus provide a higher level of protection, which is useful because biothreat exposure could be involved with much higher levels of agents than natural exposure. Therefore, therapeutic antibodies can provide

higher-than-natural protection. In addition, therapeutic antibodies have substantial advantages over antimicrobial drugs, such as high target specificity, lower toxicity, and long serum half-life (around 20 days). Antibodies can be used for both pre- and post-exposure protection against biothreat agents [5, 6].

Antibody therapy against infectious diseases in humans was first developed a century ago [7, 8], when German physiologist, Emil Adolf von Behring discovered an effective therapeutic use of serum against diphtheria. He was then awarded the first-ever Nobel Prize in Physiology or Medicine in 1901 for his contribution [9]. At that time, human hyperimmune sera obtained from convalescent donors was used to treat some infections caused by microorganisms, currently classified as biothreat agents, such as *Bacillus anthracis*, *Brucella species*, and variola virus. Therefore, antibody therapy had its beginnings as serum therapy. Serum or plasma used in such therapies contains a population of antibodies of which only a small fraction is antigen specific. In today's practise, only purified antibodies from human plasma can be approved for treatment of several infectious diseases, such as tetanus, rabies, measles, and hepatitis. Despite unquestioned efficacy in the fight against infectious diseases, human plasma-derived antibody therapy suffers from a number of clear drawbacks, such as the limited availability of donor blood, batch-to-batch variation, the risk of infectious disease transmission, and the high cost of production.

The limited availability of suitable human plasma source materials led to the development of animal plasma-derived antibodies. However, there is a major disadvantage of these products, notably serum sickness of recipients due to foreignness to humans. In other words, the animal antibody products are foreign to humans and then could elicit an immune response in humans. Repeat administration of these antibodies may result in rapid clearance of the animal antibodies to decrease the effectiveness of treatment and anaphylaxis, an allergic reaction to the animal antibodies that can range from a mild form, like a rash, to a more extreme and life-threatening response, such as renal failure [10]. A potential solution to this problem is to develop transgenic animals which carry human antibody genes. Following immunization with specific antigens, the transgenic animals will develop human antibodies [11]. The challenges for the human antibodies from transgenic animals are potential batch to batch variation, contamination with plasma-derived animal proteins, and presence of infectious pathogens.

The development of monoclonal antibodies (mAbs) by mouse hybridoma technology in the late 1970s opened a new era in antibody therapy. MAbs are monospecific, homogeneous, and reproducible [12]. The major benefit afforded by this technology is that it is possible, in principle, to develop an antibody against any target of choice and to produce it in unlimited amounts. However, like animal plasma-derived antibodies, these mAbs have a serious problem of fatal anaphylaxis in humans [13]. Using hybridoma methods to immortalize human B-cells would mitigate the problem, but the absence of a suitable fusion partner for human B cells and other technical issues have made methods that rely on human B-cell immortalization problematic. To overcome this hurdle, chimerization or humanization of murine mAbs can be implemented [14]. Modern alternative strategies now even allow development of fully human antibodies directly from phage-display libraries of human antibody fragments [15]. Another approach is to use mice that are transgenic for the human immunoglobulin (IG) locus as mentioned above [11]. Immunization of such a transgenic mouse leads to the development of human antibodies, from which hybridomas that produce human antibodies can be generated. However, due to their monospecific nature, mAbs might have some limited effectiveness against pathogens that are complex or highly mutagenic. Several different human or humanized mAbs against various

antigens on the same pathogen can be combined together to make a cocktail of mAbs to overcome the monospecific disadvantage of mAbs as therapeutics.

Although a considerable number of therapeutic mAbs are commercially available, only two mAbs have been licensed by Food and Drug Administration (FDA) for the treatment of infectious diseases [16, 17]. The high cost of antibody manufacturing in mammalian cells and relatively small market demand are the two major obstacles to widespread mAb use against infectious diseases.

The cost could be reduced by certain new technologies, such as plant-based antibody production. Plants are being evaluated for production of therapeutic antibodies at the moment, which might lead to significant progress in the near future [18]. Plant systems have several advantages over mammalian cell culture. They are fast, efficient, highly versatile (for new product development), easily scalable, and inexpensive. In addition, they are free from contamination by mammalian pathogens.

Low demand means less margin for profit, which tends to drive industry away. Pharmaceutical companies prefer devoting their resource to larger and consequently more lucrative markets, such as therapeutic antibodies against chronic conditions, such as hypertension, cancer, and heart disease, rather than infective diseases. Government intervention through funding of research or other mechanisms could improve the situation for development of therapeutic antibodies against infective diseases.

Currently, the development of antibodies for prophylaxis and treatment of biothreat agent-mediated diseases is still in its initial stages. The status of development of therapeutic antibodies against an extensive list of biothreat agents and a table (Annex A) summarizing the development stages (discovery, pre-clinical, and clinical) of each anti-biothreat agent antibody are provide in the remainder of this report.

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2 Antibodies against biothreat agents

2.1 *Bacillus anthracis*

B. anthracis is the gram-positive and spore-forming bacterium that causes, depending on the route of exposure, cutaneous, gastrointestinal, or inhalation anthrax [19]. Inhalation anthrax is the most deadly form of the disease. The 2001 anthrax attacks in the USA clearly demonstrated the severity of the hazard posed by this organism and highlighted the need for improved medical countermeasures. The virulence of *B. anthracis* is mediated by two toxins, "lethal factor" (LF) and "edema factor" (EF). Both toxins enter cells with from "protective antigen" (PA) [20]. PA is the most immunogenic of the three virulence-associated proteins and hence several PA-specific neutralizing mAbs have been developed, including two mAbs developed at DRDC Suffield [21]. Promising results have been demonstrated with a number of these mAbs *in vivo*. For example, a chimerized mAb specific to PA completely protected guinea pigs against a $40\times LD_{50}$ *B. anthracis* spore challenge following two separate 10 mg/kg administrations of the mAb, with one given before the challenge and the second on day 4 following exposure [22]. Another human mAb (referred to as MDX-1303 or Valortim) was developed from transgenic mice immunized with recombinant PA [23]. A single intramuscular injection of 1 mg/kg of MDX-1303 one hour postchallenge fully protected monkeys challenged with aerosolized Ames strain anthrax spores ($200\times LD_{50}$). Most recently, human polyclonal antibodies (pAbs) specific to PA have been developed by immunization of transchromosomic cattle carrying the human IG loci with PA [24]. When mice were challenged with 10^6 anthrax (Sterne strain) spores per mouse and given 90 mg/kg of the pAb 4 hours postchallenge, complete protection was observed, while 90% of the negative control died.

Besides PA-specific mAbs, a number of LF- or EF-specific mAbs have been developed as well. In one example, an anti-LF mAb 9A11 was demonstrated to provide significant protection to the mice when administrated as low as 0.375 mg/mouse at 24 h before anthrax lethal toxin challenge [25]. Another mAb specific to EF, EF13D, protected mice from both EF-induced footpad edema and systemic EF-mediated lethality [26].

These anti-PA, anti-EF, and anti-LF mAbs could potentially be used together to provide better protection or therapy against anthrax infection.

Currently, several human or humanized anti-PA mAbs are being developed commercially for application in pre-exposure prophylaxis and post-exposure therapy against inhalation anthrax. These include the humanized and affinity-enhanced mAb ETI204 by Elusys Therapeutics under the tradename "Anthim" [27], the human mAb1303 under the tradename "Valortim" by PharmAthene Inc. in collaboration with Medarex [28], and PamAb under the tradename "Abthrax" or "Raxibacumab" by Human Genome Sciences Inc. (HGS) [17]. Since it is unethical to conduct human studies with biothreat agents, animal studies in two species along with pharmacokinetics studies in healthy volunteers are sufficient for FDA approval [29]. All three antibodies have successfully completed these studies and received Fast-Track and Orphan Drug status from the FDA. In April 2009, HGS completed the delivery of 20,000 doses of

Raxibacumab to the US strategic national stockpile. In July 2009, HGS received a second order for 45,000 doses to be delivered over a period of three years, beginning near the end of 2009. Most recently, Raxibacumab was approved by FDA in December 2012 [17].

In addition, a human anti-PA mAb (AVP-21D9) is in clinical trial by Emergent BioSolutions [30], while a human anti-LF mAb, "Anthraxumab", was under development by IQ Corporation in the Netherlands [31].

Cangene Corporation in Winnipeg and Emergent Biosolutions in Maryland both manufacture polyclonal "Anthrax IG" (AIG) from plasma of human volunteers who have been vaccinated with anthrax vaccines [32]. Although this product is still in discovery stage, the US government has announced plans to purchase 10,000 doses of AIG manufactured by Cangene Corporation for the US national strategic stockpile.

2.2 Botulinum neurontoxin (BoNT)

BoNT, produced by *Clostridium botulinum*, is the most toxic substance known; it consists of two chains, a "heavy chain" (HC) responsible for binding to nerve cells and a "light chain" (LC) responsible for inhibition of never function [33, 34]. There are seven BoNT serotypes, A to G. BoNT is taken up by nerve cells through pinocytosis; the LC then prevents acetylcholine release from vesicles by blocking their attachment to the cell membrane, leading to muscular paralysis [35].

There are two plasma-derived antibody products marketed currently. One is a heptavalent botulinum antitoxin (H-BAT) produced by Cangene Corporation that contains antibodies specific for the seven toxin types (A–G). Although H-BAT had not yet received FDA approval, the State of Alaska recommended its use in 2010 to address a high rate of botulism [36]. H-BAT contains purified Fab derived from equines that have been immunized with botulism toxoids and toxins. The other is Botulism IG Intravenous-Human (BIG-IV or BabyBIG) developed by the California Department of Public Health [37]. BIG-IV is the only drug currently available to treat infant botulism.

Using phage display and hybridoma technology, antibody single-chain variable fragments (ScFvs) have been raised against BoNT/A [38]. Two HC specific anti-BoNT/A ScFvs each significantly prolonged the time to neuroparalysis, 1.5- and 2.7-fold, compared to the toxin control in a mouse model. When both ScFvs were used in combination, the time to neuroparalysis increased to 3.9-fold compared to the control. Furthermore, a combination therapy consisting of antibodies against both HC and LC of the toxin significantly increased protection, even at a lower mAb dosage against both pre- and post-exposure to BoNT/A holotoxin [39].

Due to the side effects of serum sickness with equine IG and mouse mAbs, the development of human antibodies to BoNTs has been explored, including one human recombinant anti-BoNT/A mAb developed at DRDC Suffield [40]. Human botulinum IG from volunteers immunized with pentavalent botulinum toxiod vaccine (A, B, C, D, and E) was demonstrated to be protective in guinea pigs against aerosol BoNT challenge [41]. Dessain's group developed two human antibodies, 4LCA and 6A, against BoNT/A and found that combining two human mAbs could completely protect mice when 50 μg of each of the 4LCA and 6A antibodies (total of 100 μg)

were first premixed with 1,000×LD_{50}s of BoNT/A and the mixture administered was intravenous injection one hour later [42]. However, 4LCA and 6A alone only partially protected mice. Similarly, Yu and coworkers developed two human antibodies using a human antibody phage display approach. Although neither antibody completely protected mice from toxin, they both prolonged the time to death when challenged with 20×LD_{50} of BoNT/A. When used together, the two mAbs completely neutralized 1,000×LD_{50} of BoNT/A/mg antibody [43]. Increasing evidence suggests that the combination of mAbs specific to different epitopes on BoNT/A can significantly improve protective function. Epitope mapping studies have identified at least three different binding sites for neutralizing antibodies on BoNT/A [44]. Subsequent combination studies confirmed that a cocktail of antibodies covering these three epitopes were 10 or 100 times more protective than the combination of the two or the individual mAb alone, respectively [45]. This cocktail was also ~100 times more efficient than hyperimmune serum.

XOMA in California is commercializing a cocktail of three human anti-BoNT/A mAbs (XOMA 3Ab) in pre-clinical studies [46]. National Institutes of Health (NIH) has awarded three contracts for a total of nearly $100 million to XOMA to develop XOMA 3Ab.

2.3 Ricin

Ricin is a 60 to 65 kDa glycoprotein derived from beans of the castor plant. It is a relatively simple toxin consisting of ricin toxin chain A (RTA) and ricin toxin chain B (RTB) linked by a disulfide bond. RTB is responsible for binding to specific sugar residues on a target cell surface, allowing RTA to be internalized into the cell by endocytosis. RTA then enzymatically inactivates the ribosome inhibiting protein synthesis irreversibly. Ricin is one of the most potent cytotoxins known. As little as a thousandth of a gram of ricin can kill an adult.

Toxin-neutralizing antibodies are potent antidotes. Several studies have demonstrated that anti-ricin antibodies can protect mice from lethal challenge of ricin [47, 48]. D9, a mAb developed at DRDC Suffield, was found to be exceptionally effective in both pre- and post-exposure efficacy assays *in vivo*. Intraperitoneal (i.p.) administration of D9, at a low dose of 5 μg per mouse, 6 hours after or 6 weeks before the challenge with 5×LD_{50} ricin was able to rescue or protect 100% of the mice [49]. D9 has been successfully humanized [50]. This appears to be the first humanized anti-ricin mAb with demonstrated therapeutic efficacy *in vivo*. It has potential both as a prophylactic for first responders and as a therapeutic for causalities of a ricin incident.

2.4 Staphylococcal enterotoxin B (SEB)

SEB is a toxin commonly associated with food poisoning [51]. It is produced by the bacterium *Staphylococcus aureus* and is known for causing toxic shock syndromes due to an SEB-related cytokine storm [52]. The symptoms, such as acute vomiting and diarrhoea, are severely incapacitating. Administration of mAbs against major histocompatibility complex (MHC) II of macrophage protected mice from lethal challenge with SEB by inhibition of enterotoxin-induced secretion of cytokines by macrophages, indicating antibodies can potentially be an effective antidote [53].

Transfer of antibodies against whole SEB toxin generated in chickens (immunoglobulin Y (IgY)) suppressed cytokine responses and was protective in mice and rhesus monkeys treated with the

IgY (10 mg/kg) up to 4 hours after challenge with a lethal SEB aerosol exposure [54]. These findings suggest antibodies raised in chickens against the holotoxin may have potential therapeutic value within a therapeutic window of opportunity after SEB intoxication.

Recently, a human anti-SEB mAb, IgG119, has shown complete protection in mice over a wide range of challenge doses of SEB (from 0.125 μg to 4 μg) when administered one hour post challenge at 200 μg/mouse [55].

2.5 Epsilon toxin

The epsilon toxin produced by *Clostridium perfringens* (a Gram-positive, anaerobic spore-forming rod bacterium) is a pore–forming protein that causes potassium and fluid leakage from cells, resulting in a severe and often fatal illness (enterotoxemia) characterized by cardiac, pulmonary, kidney, and brain edema. Some anti-epsilon-toxin neutralizing mAbs have exhibited inhibition of epsilon-toxin cytotoxicity towards cultured cells and the ability of the toxin to form pores in the plasma membranes of cells [56]. There has not yet been any report of efficacy *in vivo*.

2.6 Shiga toxin

Shiga toxins are a family of related toxins within two major groups, Stx1 and Stx2. The most common sources for Shiga toxin are the bacterium *Shigella dysenteriae* and the Shigatoxigenic group of *Escherichia coli* (STEC), which includes serotypes O157:H7, O104:H4, and other enterohemorrhagic *E. coli* (EHEC). The toxin requires highly specific receptors on a cell's surface in order to attach and enter the cell. Species such as cattle, swine, and deer that do not carry these receptors can harbour toxigenic bacteria without any ill effect, shedding them in their feces, which can then contaminate foods and spread to humans.

One report demonstrated that IgY, extracted from egg yolk of chickens immunized with Stx2, was able to recognize Stx2. This anti-Stx2 IgY effectively blocked the biological activity of Stx2 on Vero cells and protect mice from Stx2 challenge. The data suggest that anti-shiga antibodies could be antidotes against shiga toxin [57].

2.7 *Yersinia pestis*

Y. pestis is a Gram-negative bacterium and the causative agent of plague, one of the world's most deadly infectious human diseases. It infects through fleabites, direct ingestion, or inhalation of contaminated animal tissues. Two immunogenic antigens have been identified, F1 and V antigens. Horse hyperimmune serum was employed to treat human plague in the pre-antibiotic era, resulting in reduced mortality [58]. MAbs against F1 and V antigens have also been demonstrated to protect mice against lethal infection [59]. When F1- and V-specific mAbs were administered together, a significant synergistic effect was observed, demonstrating 1,000 times greater efficacy than either antibody alone [60]. Importantly, mAb administration also protected against experimental pneumonic plague.

2.8 Variola virus

Smallpox is an infectious disease unique to humans; transmission is generally through inhalation of airborne variola virus. It is considered one of the most dangerous potential biothreat agents because it is easily transmitted from person to person and no effective therapy exists. Although a worldwide immunization program eradicated smallpox disease in 1977, small quantities of smallpox virus still exist in two secure facilities in the United States and Russia.

In the early 20th century, administration of convalescent-phase serum was used to treat patients with smallpox infection. The experience with the use of vaccinia virus vaccine to prevent smallpox indicates that the antibodies induced by vaccinia virus vaccine are cross-protective for variola virus. Neutralizing and protective antibodies to vaccinia virus bind to viral envelope antigens. Most importantly, administration of vaccinia hyperimmune IG to persons in close contact with smallpox patients substantially reduced the incidence of smallpox disease [61].

Currently human Vaccinia Immune Globulin (VIG) developed by Cangene Corporation is the only antibody product licensed by FDA and Health Canada [30]. VIG contains specific antibodies to the vaccinia virus with limited potency [62].

Two human recombinant antibodies against H3 and B5 proteins on the virus surface, hV26 and h101, respectively, were developed from transgenic mice and have been demonstrated to provide 50% protection to mice when administered one day before vaccina virus challenge [63].

2.9 Intracellular bacteria

The intracellular pathogens, by their very nature, cause diseases after invading and growing in host cells. Antibodies are naturally secreted into extracellular space and binds antigen extracellularly to exert their antimicrobial functions. The currently accepted view is that antibodies do not enter cells. Therefore, an accepted paradigm is that antibodies play little role in protection against intracellular bacteria. However, there is growing evidence demonstrating that antibody-mediated immunity provides crucial protection against intracellular bacteria, as shown below. Possible mechanisms may include these antibodies interacting with the bacteria before these enter cells, during cell-to-cell spread of the pathogen, or the antibodies entering the mammalian cells after the invasive bacteria have done so.

2.9.1 *Burkholderia pseudomallei* and *Burkholderia mallei*

B. pseudomallei and *B. mallei* are closely related Gram-negative, bipolar, aerobic bacteria that can cause serious diseases, namely, melioidosis and glanders, respectively, in humans and animals [64]. Even with aggressive antibiotic treatments, the mortality rates of patients with melioidosis or glanders are very high and there is no effective vaccine for either pathogen. Consequently, more effective measures for the prevention and treatment of these diseases are urgently required.

Many mouse mAbs against *B. pseudomallei* and *B. mallei* have been reported, but very few of them showed efficacy *in vivo* [65, 66]. For example, mAbs BP7 2C6 and BP7 2G6 showed complete prophylactic protection against *B. pseudomallei* challenge and mAbs BP 2E7 and BP

2F4 showed complete prophylactic protection against *B. mallei* challenge [67]. These mAbs are specifically against either the capsular polysaccharides (PS) or the lipopolysaccharides (LPS) of the bacteria. However, the mAbs themselves did not show any direct inhibition of *Burkholderia* bacteria. These did show strong bactericidal effects against *Burkholderia* bacteria, but only in the presence of complement and phagocytes, indicating that these bactericidal effects are mediated through antibody effector functions, which are only extracellularly available and these antibodies might not be efficacious against bacteria once within cells.

2.9.2 *Francisella tularensis*

F. tularensis, the causative agent of tularaemia, is a Gram-negative intracellular bacterium with several subspecies. It infects humans through the airways, gastrointestinal tract, and skin. Immune serum treatment provided 100% prophylactic protection against intranasal lethal challenge of *F. tularensis* live vaccine strain (LVS) infection in mice while normal serum or IG-depleted immune serum provided no protection when immune serum was given 2 hours pre-exposure [68]. Furthermore, the protective efficacy with antibodies was found to be correlated with expression of FcR on phagocytes activated by interferon-gamma [69], suggesting that a critical interaction of antibody-mediated and cell-mediated immune responses is necessary to provide sterilizing immunity against *F. tularensis*. Of considerable interest was the finding that antibodies were capable of conferring protection against lethal respiratory tularemia in mice when given 24–48 hours post exposure [70].

2.9.3 *Brucella*

Brucella is a genus of Gram-negative bacteria. They are small, non-motile, and non-encapsulated coccobacilli that function as facultative intracellular parasites to cause brucellosis. The disease is transmitted by ingesting infected food, direct contact with an infected animal, or inhalation of aerosols. Antibodies specific for the O PS (OPS) or M epitopes of *Brucella* were protective in mice [71, 72]. A variety of different murine mAbs to *Brucella* have demonstrated protective efficacy in mice against experimental brucellosis [73]. These results indicate the existence of multiple antigens in *Brucella*, which can elicit protective antibody responses.

2.9.4 *Coxiella burnetii*

C. burnetii is the causative agent of Q fever. Transfer of antibodies from immunized mouse serum was protective against murine experimental infection with *C. burnetii* [74]. This protection was effective in helping to clear murine infection only if given before or at the same time as a challenge with *C. burnetii*. Of note, antibody transfer was not effective in T-cell-deficient mice, indicating that intact cell-mediated immunity is needed for antibody function [75].

2.10 Encephalitis viruses

Viral encephalitis can be caused by a number of viruses, including eastern equine encephalitis virus (EEEV), Venezuelan equine encephalitis virus (VEEV), western equine encephalitis virus (WEEV), Japanese encephalitis virus (JEV), and West Nile virus (WNV), which are of concern as possible bioterrorism agents or emerging public health concerns.

Development of neutralizing anti-VEEV antibodies has been extensively explored. Protective anti-VEEV mAbs provided protection against lethal challenge in the experimental murine model. Among these, three have been successfully humanized [14, 76, 77]. A humanized anti-VEEV antibody developed at DRDC Suffield provided complete protection to mice against VEEV infection 24 hours before or after virulent VEEV challenge, indicating that this antibody has potent prophylactic and therapeutic effects against VEEV [14].

In the case of JEV, studies have shown that transfer of mouse mAbs can protect against prior or subsequent JEV exposure in mice, goats, and monkeys [78, 79]. Humanized anti-JEV mAbs exhibited high neutralizing activities against a broad spectrum of JEV genotype strains in an *in vivo* mouse encephalitis model [80].

Available evidence suggests that WNV might be more susceptible to antibody-mediated than cell-mediated immunity. In a mouse lethal WNV infection model, human IG from convalescent donors showed a significant protective effect in both prophylaxis and therapy against WNV [81]. For example, treatment of mice with 0.2 or 1 mg human IG after virus infection led to 100% survival. A potent neutralizing anti-WNV humanized mAb (hE16) demonstrated improvement in the course of disease in a hamster model when administered after the virus had infected neurons in the brain [82]. Another human ScFv (CR4374) showed 50% protection in mice from lethal WNV challenge at the dose of 12.9 μg/kg of body weight [83].

2.11 Hemorrhagic fever viruses (HFVs)

HFVs include the filoviruses (Ebola, Marburg), arenaviruses (Lassa), bunyaviruses (Hanta), and flaviviruses (Dengue). The resulting diseases are characterized by fever and bleeding disorders that can progress to high fever, shock, and death in extreme cases.

Antibodies from convalescent serum have been used for treatment of Ebola, Marburg, and Lassa hemorrhagic fevers in patients with curative effects. The protective effectiveness of human anti-Ebola virus (EBOV) serum IG has been confirmed in mice and monkeys [84, 85]. Moreover, some mAbs to EBOV have demonstrated protection for the mice from EBOV infection *in vivo* [86]. Subsequently, a neutralizing human mAb to EBOV glycoprotein, KZ52, protected guinea pigs from lethal EBOV challenge. Administration before or up to 1 hour after challenge resulted in dose-dependent protection by the antibody [87].

Anti-Marburg virus (MBGV) convalescent sera from guinea pigs showed some protection in mice [88]. In addition, some mAbs to MBGV glycoprotein provided substantial, but incomplete, protection of naive guinea pigs by antibody transfer [89]. These data suggest that neutralizing epitopes exist within the MBGV glycoprotein but that induction of antibodies to these neutralizing epitopes may not be sufficient for protection from lethal infection.

Anti-Hanta virus (HTNV) mAb, which recognizes G2 envelope glycoprotein, provided protection in mice from HTNV infection [90]. The results indicate that the anti-HTNV mAb has anti-HTNV activity *in vivo* and could be an effective candidate for the treatment of patients infected by HTNV. Some anti-HTNV mAbs are in clinical stages of testing (I/II) in China [90, 91].

There are four dengue virus (DV) serotypes that are very close in terms of antigenicity. MAbs against different DV serotypes have been developed, some of which were protective in mouse

models [92, 93]. While antibodies can play a vital protective role during DV infection, they have also been linked to some problems, such as antibody-enhancing infectivity in DV infection. It has been noticed that infection with one serotype is thought to protect against re-infection with the same serotype, but may either protect against or enhance infection with one of the other three serotypes. The increased severity of secondary infections of heterologous seroptypes is believed to result from antibody dependent enhancement (ADE) of DV infection, in which FcR engagement by antibody-virus immune complexes facilitates virus entry into susceptible myeloid cell types. Once inside white blood cells, the virus replicates undetected, eventually generating very high virus titers which cause severe disease. Administration of a high dose of DV serotype-specific antibodies eliminated viremia, but administration of lower doses enhanced DV infection *in vivo* even when the antibody possessed neutralizing function *in vitro* [94]. By contrast, a genetically engineered antibody (E60-N297Q) that cannot bind FcR exhibited prophylactic and therapeutic efficacy against ADE-induced DV lethal challenge [94]. In addition, a humanized anti-DV4 antibody with a nine-amino-acid deletion in the Fc region completely ablated ADE of DV replication *in vitro*. Antibody transfer of this humanized antibody at 20 μg/mouse afforded 50% protection of suckling mice against challenge with $25\times LD_{50}$ of DV4. Monkeys that received 2 mg/kg of body weight of this antibody were completely protected against $100\times LD_{50}$ of DV4 [95].

These observations provide insight into the pathogenesis of ADE dengue disease and identify a novel strategy for the design of therapeutic antibodies against dengue.

2.12 Emerging viral diseases

2.12.1 Severe acute respiratory syndrome (SARS) coronavirus (SCV)

SCV first appeared in humans in 2002 and eventually infected ~8,000 people with a 10% fatality rate. Within weeks, SARS spread from China to 37 other countries around the world. SCV infection leads to the generation of potent neutralizing antibodies. For example, antibody transfer of hyperimmune serum to naïve mice prevented virus replication following SCV challenge [96]. Patients with SARS were also treated successfully with IG from convalescent patient plasma [97]. Meanwhile, several research groups have recently developed human mAbs to the SCV spike glycoprotein. One of these, developed from immortalized human B-cells, neutralized the virus 300-fold more efficiently than convalescent serum [98]. In a mouse model of SCV infection, this antibody prevented viral replication. When a second human mAb, derived from the human ScFv library, was given prophylactically to mice at doses therapeutically achievable in humans, viral replication was reduced below assay limits [99]. A third human mAb selected from another large antibody library showed neutralizing activity in ferrets [100]. A fourth human antibody, 5H10, inhibited propagation of the virus and pathological changes in rhesus macaques infected with the virus through the nasal route. The mechanism of therapy was by the inhibition of fusion between the virus envelope and host cell membrane [101]. Two other human mAbs derived from transgenic mice with human IG genes completely protected mice from viral replication in the lung [102]. More human mAbs to SCV are being developed.

2.12.2 Avian influenza H5N1 virus

H5N1 virus poses a significant potential threat were human–human transmission to develop, with the possibility of a Spanish flu-like pandemic [103]. Eleven outbreaks of H5N1 were reported worldwide in June 2008 in five countries, compared to 65 outbreaks in June 2006 and 55 in June 2007. Anti-influenza antibody therapy represents an alternative paradigm for preventing or treating viral infection. Evidence for the utility of anti-influenza antibodies dates back to the 1918 influenza pandemic, when serum transfer was used with some success [104]. Although protection provided by anti-influenza mAbs is typically narrow in breadth because of the antigenic heterogeneity of influenza viruses, several groups have recently reported human protective mAbs that bind to conserved epitopes within the stem region of viral hemagglutinin (HA) [105–107]. However, these epitopes appear to be restricted to a subset of influenza viruses, not for all. Just recently, a previously unknown conformational epitope within the ectodomain of the influenza matrix 2 protein, M2e has been found. This epitope is highly conserved in influenza A viruses, being present in nearly all strains known to date, including highly pathogenic viruses that infect primarily birds and swine and the 2009 swine-origin influenza virus A H1N1 pandemic strain. In addition, human anti-M2e mAbs have been found to protect mice from lethal challenges with either H5N1 or H1N1 influenza viruses [108]. These results suggest that viral M2e may elicit broadly cross-reactive and protective antibodies in humans.

2.12.3 Chikungunya virus (CHIKV)

CHIKV is a recently re-emerged arbovirus responsible for a massive outbreak of infection in the Indian Ocean region. Infection by CHIKV is often characterized by long-lasting and incapacitating arthritis. Some fatal cases have been described among elderly and newborns. Currently, there is no available vaccine or specific treatment against CHIKV. A clinical study demonstrated that IgG3 response was dominated in patients infected with CHIKV and early high levels of anti-CHIKV IgG3 indicated a favorable prognosis [109]. Human IG from convalescent plasma of CHIKV infection exhibited a high *in vitro* neutralizing activity and a powerful prophylactic and therapeutic efficacy against CHIKV infection *in vivo* [110]. To date, two human mAbs against CHIKV, 5F10 and 8B10, have been developed from human blood B-cells of a donor with a history of CHIKV infection. Both of these not only neutralized different CHIKV isolates from Singapore, Africa, and Indonesia *in vitro*, but also the closely related O'nyong-nyong virus [111]. Both were specific for the CHIKV envelope: 5F10 bound to the E2 glycoprotein ectodomain and 8B10 to E1 and/or E2.

2.12.4 Hendra virus (HeV) and Nipah virus (NiV)

HeV and NiV are recently-emerged, closely related and highly pathogenic paramyxoviruses. Both can cause fatal disease in animals and humans. Development of mAbs specific for these viruses has only recently started, but is progressing rapidly. Antiserum transfer protected against a NiV challenge in hamsters [112]. Furthermore, animals that received anti-NiV murine mAbs developed from NiV envelope proteins, G attachment protein, or F fusion protein, either before or immediately following NiV challenge, were completely protected [113]. Interestingly, mAb specific for the NiV protein neutralized HeV *in vitro* and efficiently protected hamsters from HeV if given before infection [114]. These results reveal the similarities between HeV and NiV pathogenesis, particularly in affecting both respiratory and neuronal system. In 2009, human

mAbs against both viruses were developed [115]. One of these, m102.4, which targeted the ephrin-B2 and -B3 receptor binding domain of the G envelope glycoprotein of HeV and NiV, showed exceptional cross-reactive potency against both NiV and HeV *in vitro*. It also has confirmed efficacy against lethal challenge of NiV in a new ferret model [116] and HeV in an African green monkey model [117].

2.12.5 Crimean-Congo hemorrhagic fever virus (CCHFV)

CCHFV, a member of the genus *Nairovirus* of the family *Bunyaviridae*, causes a severe disease in humans with a high mortality rate. Found in Europe, Africa, and Asia, CCHFV is a re-emerged, tick-borne virus. There is currently no specific antiviral therapy for CCHF. Advances in the development of mAbs and antibody engineering have raised hopes for new candidate drugs for the prevention and treatment of CCHFV infections. In Turkey, the treatment of CCHF victims with human CCHFV IG from convalescent plasma of CCHFV infected patients has been evaluated. The survival rate was found to be 86.6% (13/15), although two patients died despite CCHFV IG administration, indicating prompt administration of CCHFV IG might be a very promising new treatment approach, especially for high-risk individuals [118].

3 Summary

This technical memorandum has reviewed the state of development of therapeutic antibodies against a wide range of biothreat agents and emerging diseases that potentially pose operational threats to the Canadian Armed Forces (CAF). In the case of anthrax, effective therapeutic antibodies have already entered production and others are in advanced stages of development. Against some other threat agents, in particular, intracellular parasites such as *Burkholderia pseudomallei* (agent HI), the causative agent of melioidosis, candidate therapeutic antibodies have shown meagre efficacy and effective antibodies might never be developed. Nonetheless, with continuous advances in the technology of antibody development and understanding of the infectivity and intoxication of biothreat agents, more and more antibodies will likely be developed as effective prophylactic and therapeutic products in the near future. Specific antibodies may be the only hope for the numerous biothreat agents against which no vaccines and drugs are currently available.

DRDC Suffield, in particular, has significant strength in antibody research and development. It has established a series of novel approaches to developing antibodies, ranging from mAbs through to human or humanized antibodies. Using these approaches, a number of human or humanized antibodies, such as those against VEEV [14, 119], anthrax, botulinum neurotoxin A [40], and ricin [50] have been developed. The most recently developed humanized anti-ricin antibody is the most efficacious anti-ricin antibodies yet reported. To exploit its potential, the Canadian Forces Health Services are providing financial support from under the Biological Warfare Threat Medical Countermeasures project to move this antibody to the preclinical development stage. The United States National Institutes of Health are also considering providing NIH-funded services for further development of this antibody.

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Annex A Development status of anti-biothreat agent antibodies

Biothreat agent	Antibody	Target	Stage of development	Company	Indication
B. anthracis	Anthin (humanized mAb) [27]	PA	FDA approval pending	Elusys Therapeutics	Prevention & treatment of anthrax
	Valortim (human mAb) [23, 28]	PA	FDA approval pending	PharmAthene	Prevention & treatment of anthrax
	Abthrax or Raxibacumab (human mAb) [17]	PA	Approved	HGS	Prevention & treatment of anthrax
	AVP-21D9 (human mAb) [30]	PA	Phase I	Emergent BioSolutions	Prevention & treatment of anthrax
	Human pAbs [24]	PA	Discovery		Protective in mice
	AIG from hyperimmune human plasma [32]	Whole cells	Phase I	Cangene & Emergent BioSolutions	Prevention & treatment of anthrax
	Anthraxumab (human antibody) [31]	LF	Preclinical	IQ Corporation	Protective in mice
	Murine mAb [23]	EF	Discovery		Protective in mice
BoNT	H-BAT, heptavalent IG from equine hyperimmune plasma [32]	BoNT/A-G	FDA approval pending	Cangene	Prevention & treatment of botulism A to G
	BIG-IV or BabyBIG from human hyperimmune plasma [37]	BoNT/A,B	Approved	California Department of Public Health	Treatment of infant botulism types A & B
	XOMA 3Ab, a cocktail of 3 human mAbs [46]	BoNT/A	Preclinical	XOMA	Protective in mice
Ricin	Murine mAbs [47–49]	Holotoxin, chain A, or chain B	Discovery	Including DRDC	Protective in mice
	Humanized antibodies [50]	Chain B	Preclinical	DRDC	Protective in mice

Biothreat agent	Antibody	Target	Stage of development	Company	Indication
SEB	Murine mAb [53]	MHC of macrophage	Discovery		Protective in mice
	Chicken hyperimmune IG [54]	Holotoxin	Discovery		Protective in mice
	Human mAbs [55]	Holotoxin	Discovery		Protective in mice
Epsilon-toxin	Murine mAbs [56]	Membrane insertion domain of the toxin	Discovery		Protective *in vitro*
Shiga toxin	Chicken IgY [57]	Stx2	Discovery		Protective in mice
Y. pestis	Mouse mAbs [59, 60]	F1 and V	Discovery		Protective in mice
Variola	VIG (human IG from human hyperimmune plasma) [32]	Vaccinia virus	Approved	Cangene	Protection & treatment of smallpox
B. pseudomallei & B. mallei	murine mAbs [67]	PS or PLS	Discovery		protective in mice
F.tularensis	Murine serum [70]	LVS	Discovery		Protective in mice
Brucella	Murine mAbs [71, 72]	OPS or M epitopes	Discovery		Protective in mice
C. burnetii	Murine serum [74]	Whole cells	Discovery		Protective in mice
VEEV	Recombinant humanized mAbs [14, 76, 77]	E2	Discovery	Including DRDC	Protective in mice
JEV	Murine or humanized mAbs [78–80]	Virus	Discovery		Protective in mice, goats & monkeys
WNV	hE16 (humanized mAb) [82, 120]	Domain III, E	Phase I	Macrogenics	Protective in hamsters
	CR4374 (humanized ScFv) [83, 120]	Domain III, E	Pre-clinical	Crucell	Protective in mice
EBOV	human mAb [87]	Glycoprotein	Discovery		Protective in guinea pigs
MBGV	Murine mAbs [89]	Glycoprotein	Discovery		Protective in guinea pigs
HTNV	Murine mAbs [90, 91]	G2	Clinical trials I/II	China	Protective in mice

Biothreat agent	Antibody	Target	Stage of development	Company	Indication
DV	Murine mAbs, murine Fab, or humanized mAb [92–95]	Virus	Discovery		Protective in mice and/or monkeys
SCV	Human mAbs [98–102, 121]	Spike protein	Discovery or pre-clinical		Protective in mice, ferrets, or macaques.
Avian influenza H5N1	Human mAbs, chimeric mAbs, or human ScFv [105–108]	Hemagglutinin or M2e	Discovery		Protective in mice
CHIKV	Human IG from convalescent plasma [110]		Discovery		Protective in two mouse models
	Human mAbs [111]	E1 and/or E2	Discovery		Protective *in vitro*
HeV & NiV	Human mAbs [115–117]	Protein G	Discovery		Protective in ferrets or monkeys
CCHFV	Human IG from convalescent plasma [118]	Virus	Discovery		Protective in humans

List of symbols/abbreviations/acronyms/initialisms

Ab	Antibody
ADCC	Antibody-dependent cellular cytotoxicity
ADE	Antibody dependent enhancement
AIG	Anthrax IG
BIG	Botulism IG
BIG-IV	Botulism IG intravenous-human
BoNT	Botulinum neurotoxin
CCHFV	Crimea-Congo hemorrhagic fever virus
CAF	Canadian Armed Forces
CDC	Complement-dependent cytotoxicity
CHIKV	Chikungunya virus
DND	Department of National Defence
DRDC	Defence Research & Development Canada
DV	Dengue virus
EBOV	Ebola virus
EEEV	Eastern equine encephalitis virus
EF	Edema factor
EHEC	Enterohemorrhagic *E. coli*
Fab	Antigen-binding fragment
Fc	Crystallisable fragment
FcR	Crystallisable fragment receptor
FDA	Food and Drug Administration
HA	Hemagglutinin
H-BAT	Heptavalent botulinum antitoxin
HC	Heavy chain
HeV	Hendra virus
HFV	Hemorrhagic fever virus
HGS	Human Genome Sciences Inc.
HTNV	Hanta virus

IG	Immunoglobulin
IgY	Immunoglobulin Y
i.p.	Intraperitoneal
JEV	Japanese encephalitis virus
LC	Light chain
LD_{50}	Lethal dose 50
LF	Lethal factor
LPS	Lipopolysaccharides
LVS	Live vaccine strain
mAb	Monoclonal antibody
MHC	Major histocompatibility complex
NIH	National Institutes of Health
NiV	Nipah virus
OPS	O polysaccharide
PA	Protective antigen
pAb	Polyclonal antibody
PS	Polysaccharides
R&D	Research & Development
RTA	Ricin toxin A chain
RTB	Ricin toxin B chain
RSV	Respiratory syncytial virus
SARS	Severe acute respiratory syndrome
ScFv	Single chain variable fragment
SCV	SARS coronavirus
SEB	Staphylococcal enterotoxin B
STEC	Shigatoxigenic group of *E. coli*
VEEV	Venezuelan equine encephalitis virus
VIG	Vaccinia immunoglobulin
WEEV	Western equine encephalitis virus
WNV	West Nile virus

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Antibodies have great potential for countering biothreat agents that are not addressed by current medical countermeasures. They are highly versatile defence molecules naturally produced in the body and can be developed against any biothreat agent. Antibodies can directly neutralize biothreat agents and/or invoke destruction of them through the assistance of the immune system. Unlike vaccines, which require time to induce protective immunity and depend on the host's ability to mount immune responses, therapeutic antibodies can confer instant and consistent immunity against biothreat agents once administered regardless of the immune status of the host. In addition, therapeutic antibodies have substantial advantages over antimicrobial drugs, such as high specificity, low toxicity, and long serum half-life (around 20 days). Therapeutic antibodies can be used, either prophylactically to prevent diseases before, or therapeutically to treat diseases after biothreat attacks. It is these properties that make antibodies attractive for medical countermeasures against biothreat agents. As reviewed here, the development of therapeutic antibodies against biothreat agents is summarized in the following areas: history of using antibodies against infectious diseases, current status of development of therapeutic antibodies against an extensive list of biothreat agents, and a table summarizing the development stages (discovery, pre-clinical, and clinical) of each anti-biothreat agent antibody.

Les anticorps sont des outils très prometteurs pour contrer les agents de menace biologique non neutralisés par les contre-mesures médicales actuelles. Ce sont des molécules de défense très polyvalentes naturellement fabriquées par l'organisme et qu'on peut mettre au point contre tout agent de menace biologique. Les anticorps peuvent directement neutraliser les agents de menace biologique ou amener leur destruction par le système immunitaire. Contrairement aux vaccins, qui nécessitent un délai pour induire une immunité protectrice et qui dépendent de la capacité de l'hôte à bâtir une réponse immunitaire, une fois administrés, les anticorps thérapeutiques confèrent une immunité instantanée et constante contre un agent de menace biologique, quel que soit l'état immunitaire de l'hôte. En outre, les anticorps thérapeutiques présentent des avantages appréciables par rapport aux antimicrobiens, par exemple une grande spécificité, une faible toxicité et une longue demi-vie sérique (environ 20 jours). Les anticorps thérapeutiques peuvent être utilisés dans un but prophylactique, pour prévenir les maladies, ou dans un but thérapeutique, pour traiter les maladies après une attaque par un agent biologique. Ce sont ces propriétés qui rendent les anticorps si intéressants comme contre-mesures médicales en présence d'agents de menace biologique. Dans le présent document, divers aspects de la mise au point d'anticorps thérapeutiques contre les agents de menace biologique sont résumés : historique du recours aux anticorps pour lutter contre les maladies infectieuses, état actuel de la mise au point d'anticorps thérapeutiques dirigés contre de nombreux agents de menace biologique et tableau résumant les étapes de la mise au point (découverte, essai préclinique et essai clinique) de chacun des anticorps dirigés contre un agent de menace biologique.

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antibodies; biotheat agents; biological warfare agents; emerging diseases; medical countermeasures

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