Therapeutic Antibodies for Biodefense

9

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Abstract

Diseases can be caused naturally by biological agents such as bacteria, viruses and toxins (natural risk). However, such biological agents can be intentionally disseminated in the environment by a State (military context) or terrorists to cause diseases in a population or livestock, to destabilize a nation by creating a climate of terror, destabilizing the economy and undermining institutions. Biological agents can be classified according to the severity of illness they cause, its mortality and how easily the agent can be spread. The Centers for Diseases Control and Prevention (CDC) classify biological agents in three categories (A, B and C); Category A consists of the six pathogens most suitable for use as bioweapons (*Bacillus anthracis*, *Yersinia pestis*, *Francisella tularensis*, botulinum neurotoxins, smallpox and viral hemorrhagic fevers). Antibodies represent a perfect biomedical countermeasure as they present both prophylactic and therapeutic properties, act fast and are highly specific to the target. This review focuses on the main biological agents that could be used as bioweapons, the history of biowarfare and antibodies that have been developed to neutralize these agents.

Keywords

Bioterrorism · Antibodies · Prophylaxis · Therapeutic · Biodefense · Bioweapon · Biowarfare agent · Toxin · Virus · CBRN

9.1 Introduction

9.1.1 Definitions

Various definitions of biological agent, biological weapon (bioweapon) and bioterrorism are proposed by international organizations (World Health Organization, Centers for Diseases Control and Prevention) and States.

A **biological agent** could be defined as (i) a micro-organism (natural or genetically engineered) (ii) a cell culture (iii) a human endoparasite (iv) a toxin that can induce a reaction, allergy or poisoning in a host (human or not) [64].

A **biowarfare agent** could be defined as a biological agent that is conducive to the development of a bioweapon. In the context of terrorism, the term **biothreat agent** could also be used.

A **bioweapon** could be defined as an intentionally harmful combination of one or more biological agents with a vector (bomb, rocket, nebulizer, animal, etc.). A bioweapon could be

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used directly to incapacitate or kill a single person or population, or indirectly to destabilize a nation by targeting livestock and agriculture [67].

A **danger** could be defined as any kind of damage that could affect a system (e.g. human, country, institution…).

A **threat** could be defined as a factor increasing the risk.

A **risk** could be defined as the probability of exposition of a system to a danger. The risk is increase by the threat.

Biological risk could be defined as the risk of intentional utilization of a bioweapon to harm human beings—directly or not—by means of a **threat** (e.g. State, terrorist). The notion of risk is directly connected to the notion of **vulnerability** (e.g. absence of control of the food and water supply, absence of ways of detecting biological agents, high urban population density, etc.). Once a risk is identified it may be possible to **neutralize** it (e.g. human smallpox was eradicated by mass immunization).

The **vulnerability** could be defined as the possibility for any system to be damaged by a danger.

Countermeasures are tools or devices used to prevent or to mitigate the impact of the danger (Fig. 9.1).

9.1.2 Classification

Several classification systems for biowarfare agents have been proposed by States and international organizations (World Health Organization, Centers for Disease Control and Prevention, etc.). Certain biowarfare agents are considered as a priority for biodefense in almost of these classifications but, there are some differences between the various systems because there is no consensus on selection criteria and some threats are specific to certain countries or geographical areas.

In 1949, the U.S. military microbiologist Theodor Rosebury proposed a list of ten criteria to take into account when weighing up the potential of any biological agents for biowarfare (in order of importance): [20, 102]

- Infectivity: the biological agent should be highly infective;
- Casualty effectiveness: the biological agent should be virulent and able to cause fatal or incapacitating disease in a short time frame;
- Availability: the biological agent should be easy and cheap to produce in bulk;
- Resistance: the biological agent should be able to persist in the environment (heat, ultraviolet, sunlight-radiation, disinfectant, etc.) for long enough to infect the target population. If a biological agent is not naturally resistant enough, it might be possible to engineer it;
- Mean of transmission: the biological agent should be easy to disseminate in the environment (ideally by nebulization);
- Epidemicity: depending on the desired outcome, the biological agent could be highly contagious or not. If a biological agent is highly contagious, the risk is that the aggressor could also be contaminated ("boomerang effect");

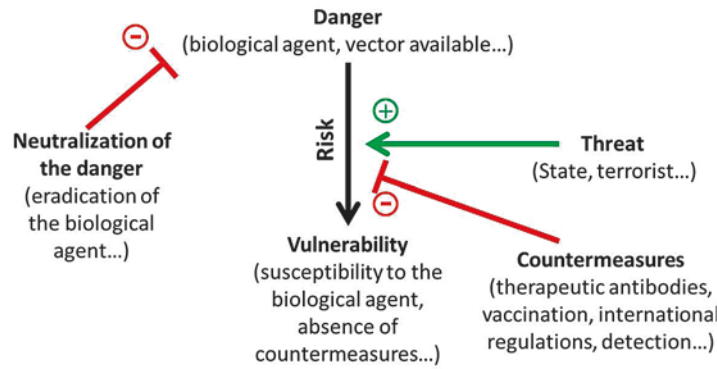

Fig. 9.1 Relationship between risk, threat and vulnerability
Green arrow: positive effect on the system.
Red arrows: negative effect on the system

- Specific immunization: the target population should not be naturally immunized against the agent or have access to a vaccine (although the aggressor should have a vaccine). Any bacterium should be resistant to conventional antibiotics;
- Therapy: the aggressor should have access to protections against the biological agent (preventive or curative treatment, individual protection equipment, etc.) to prevent a boomerang effect. No therapy should be available for the target population;
- Detection: defenders will find it more difficult to respond if it is difficult to detect—diagnose—the biological agent or the source of contamination,;
- Retroactivity: the capacity of a bioweapon to backfire on the aggressor will affect whether a nation decides to use it or not.

If bulk production of the biological agent is cheap, more countries will be able to develop the bioweapon.

The ten criteria of Theodor Rosebury were defined for the identification of a biological agent for military purposes but they are as relevant to bioterrorism, although other criteria also come into play in this context:

- The biological agent should be easily available—illegally bought, stolen or found in nature—to terrorists.
- Production of the biological agent should be easy and cheap with minimal resources;
- Resultant mortality or degree of incapacity should be high;
- Treatments should not be available, even for the terrorists (kamikazes);
- The delay between contamination and the onset of symptoms could be long, to complicate investigation and identification of the source of contamination;
- The choice of biological agent should take into account the terror that will be elicited in the population. The resulting panic would destabilize the economy and the normal function of the State.

The main classification system used for biological agents of interest for biodefense is that of the Centers for Disease Control and Prevention (CDC) (Tables 9.1 and 9.2) [22]. Two main criteria were used for this classification, namely ease of spread of the biological agent and the severity/lethality of the illness caused. Category A contains the six biological agents of major concern for biodefense; Category B lists 12 agents that are considered of secondary potential; Category C agents are those that are considered as emerging-threats for biodefense.

9.1.3 History of Bioterrorism

9.1.3.1 Use of Biological Agents in the Military Context

Bioweapons could be far more deadly than chemical or nuclear weapons, e.g. it has been estimated that 10 g of anthrax could kill as many people as a metric ton of sarin [113]. In the last century, more than 500 million people died of naturally spread infectious diseases but tens of thousands also died as a result of the deliberate release of a biological agent (Table 9.3). Paradoxically, at the same time as humankind developed drugs and systems to cure sick people, it developed bioweapons in parallel. This could be because the knowledge required to develop drugs and vaccines against pathogens can be misused to develop bioweapons. Establishing exactly when bioweapons have been used is difficult because of lack of information and the absence of formal evidence. Similarly, estimating mortality following a biological attack is complicated because it can be difficult to differentiate deaths due to a natural epidemic from those directly resulting from the attack.

The first utilization of a bioweapon seems to go back to 1350 BCE when Hittites (an ancient people who lived in Anatolia) left sick animals in villages they had plundered to infect the local population. Recent analyses reveal that the animals had died of tularemia which is in CDC Category A [123].

Table 9.1 CDC Class A, B and C biological agents

	Category A	Category B	Category C
Properties	Easily spread or transmission from person to person	Moderately easy to spread	Easily available
	High mortality rates	Induce moderate illness rates and low mortality rates	Easy to produce and spread
	Major impact for public health	Require specific enhancements of CDC's laboratory capacity and enhanced disease monitoring	Potential for high morbidity and mortality rates and major health impact
	Cause public panic and social disruption		
	Require special action for public health preparedness		
Bacteria	Anthrax (*Bacillus anthracis*)	Brucellosis (*Brucella* species)	Some multidrug-resistant bacteria (e.g. *Mycobacterium tuberculosis*)
	Plague (*Yersinia pestis*)	Epsilon toxin of *Clostridium perfringens*	
	Tularemia (*Francisella tularensis*)	Food safety threats (e.g. Salmonella species, *Escherichia coli* O157:H7, *Shigella dysenteriae*…)	
		Water safety threats (e.g. *Vibrio cholerae*…)	
		Glanders (*Burkholderia mallei*)	
		Melioidosis (*Burkholderia pseudomallei*)	
		Psittacosis (*Chlamydia psittaci*)	
		Q fever (*Coxiella burnetii*)	
		Typhus fever (*Rickettsia prowazekii*)	
Virus	Smallpox (*Variola major*)	Viral encephalitis caused by alphaviruses (e.g. Venezuelan equine encephalitis, eastern equine encephalitis, western equine encephalitis…)	Viral emerging infectious diseases (e.g. Nipah virus, hantavirus…)
	Viral hemorrhagic fevers: filoviruses (Ebola, Marburg…) and arenaviruses (Lassa, Machupo…)		
Parasite and fungi		*Cryptosporidium parvum* (water safety threat)	
Toxines	Botulism (Clostridium botulinum toxins)	Ricin toxin (*Ricinus communis*)	
		Staphylococcal enterotoxin B	

The A and B lists are exhaustive; Category C is an open list

More information is available about an attack on the seaside city of Kaffa (currently Feodossiia, Crimean peninsula) in 1346 AD. The Mongols were besieging the city and catapulted the corpses of plague victims over the city's fortifications [62, 15, 133]. This attack had major physical and psychological impact on the citizens, leading to the town's evacuation by sea. This diaspora resulted in a five-year plague epidemic that killed about a third of the population of Europe. The direct impact of the use of plague as a bioweapon is difficult to quantify because the disease might have spread naturally as a result of wartime unhygienic conditions in Kaffa.

The first use of a virus as a bioweapon was documented in 1763 in mail sent by Henri Bourquet, a British mercenary, to Jeffrey Amherst, the British military governor of the New-France. After the defeat of the French in the Seven Year War (1754–1760), strict conditions were imposed on Amerindians who had fought with the French [2, 82]. Consequently, the Amerindians rebelled

Table 9.2 Main characteristics of the biological agents that are most likely to be used as bioweapons

Biological agent	CDC category	Infectious dose	Incubation period	Interhuman transmission	Case fatality rate in absence of treatment	Prophylaxis	Treatment (approved or IND)	Serotherapy (approved or IND)
Bacillus anthracis	A	8000–10,000 spores	2–6 days	No	10% (cutaneous anthrax) to ~100% (inhalational anthrax)	Vaccine. Antibiotics.	Antibiotics: ciprofloxacin or doxycycline plus one or two antibiotics effective against anthrax (e.g. meropenem) plus a protein synthesis inhibitor (e.g. linezolid).	Yes (monoclonals): Raxibacumab and Obiltoxaximab
Yersinia pestis	A	100–500 bacteria	1–6 days	Infectious (pulmonary plague)	50–60% or ~100% (pulmonary plague)	Antibiotics.	Antibiotics: streptomycin, gentamicin, doxycycline, ciprofloxacin and/or chloramphenicol.	No
Francisella tularensis	A	10–50 bacteria	1–10 days	No	35–65% (pulmonary tularemia)	Vaccine (IND).	Antibiotherapy: streptomycin or gentamicin.	No
Variola viruses	A	10–100 virions	7–17 days	Very infectious	1% (variola minor) – 50% (variola major)	Vaccine.	Vaccination. Chemotherapy: cidofovir (IND), ST-246 (IND) or CMX001 (IND).	Yes (polyclonal): Vaccinia immune globulin (IND)
Viral hemorrhagic fevers	A	1–10 virions	4–21 days	Very infectious	High to moderate (depend on biological agent)	1 vaccine (against yellow fever). Chemotherapy: ribavirin may be effective against *Bunyaviridae* and *Arenaviridae* (IND).	Intensive supportive care. Chemotherapy: Ribavirin (IND).	Yes: Zmapp (oligoclonal) against Ebola virus (IND). Convalescent plasma for some hemorrhagic fevers (IND).
Botulinum toxins	A	LD_{50}: 1 ng.kg^{-1} (IV) to 1 µg.kg^{-1} (I)	2 h – 3 days	No	~60%	Vaccine.	Serotherapy. Antibiotics (for wound botulism). Supportive therapy.	Yes (polyclonal): BabyBIG and BAT.
Ricin	B	LD_{50}: 1 (by injection) to 25 mg.kg^{-1} (by inhalation)	3–8 h	No	2–8%	Two IND vaccines. Mask is the best protection to prevent inhalation.	Supportive therapy.	No

(continued)

Table 9.2 (continued)

Biological agent	CDC category	Infectious dose	Incubation period	Interhuman transmission	Case fatality rate in absence of treatment	Prophylaxis	Treatment (approved or IND)	Serotherapy (approved or IND)
Staphylococcus Enterotoxin B	B	Incapaciting dose: 0,4 µg. kg^{-1}	3–12 h	No	<1% but ~80% of contaminated would be incapacited for 1–2 weeks.	No	Supportive therapy.	No
Coxiella burnetii	B	1–10 bacteria	10–40 days	No	<3%	Vaccination. Antibiotics.	Antibiotics: tetracycline, doxycycline or hydroxychloroquine.	No

The case fatality rate assumes the absence of any relevant treatment or prophylaxis (vaccination.., etc.). In the event of massive contamination, the fatality case rate would depend upon the time required for diagnosis and the possibility that it would be impossible to provide effective treatment for all victims. Fatality case rate would depend upon the route of contamination and the general state of health of the target population. Here, prophylaxis is described as urgent prophylaxis (if contamination is suspected) or long-term prophylaxis (vaccination). IND: Investigational New Drug. IV: intravenous. I: ingestion

Table 9.3 Historical uses of biological agents in biowarfare

Year	Examples of utilization of biological agents as a biowarfare agent.
~1350 B.C.	Hittites leaves animals contaminated by tularemia in the villages that they had plundered.
Fourth century B.C.	According to Greek historian Herodotus, Scythian archers infect their arrows by dipping them into decomposing cadavers.
1155	Emperor Barbarossa poisons water wells with human bodies.
1346	Mongols catapult bodies of plague victims over the fortifications of Kaffa (Feodossiia, Crimean Peninsula).
1422	The Prince Zygmunt Korybutovic hurled corpses of plague-stricken soldiers, dead cows and excrement during the siege of Karlstejn (currently in Czech republic).
1495	Spanish mix wine with blood of leprosy patients to sell to their French foe (Naples, Italy).
1650	The Polish general Kazimierz Siemienowicz fire with hollow artillery spheres filled with the with the saliva of rabid dogs.
1763	British troops give smallpox-infected blankets to the Amerindians.
1797	Napoleon floods the plains around Mantua, Italy, to enhance the spread of malaria.
1785	Tunisians threw plague-infected cothing into the Chistian-held city of La Calle (currently in Algeria).
1863	Confederates sell clothing from yellow fever and smallpox patients to Union troops, USA.

This table lists some examples of reported use of biowarfare agents. The real impact of these aggressions is unclear because it is difficult to distinguish between natural and hostile contamination

against the British and won several battles. To stop the rebellion, in 1763, the British were supposed to have distributed blankets carrying the smallpox virus (obtained from the military hospitals) to "*extirpate this execrable race*". Here again, it is difficult to know whether such blankets were really given to the Amerindians and, if they were, how many Amerindians died from smallpox. Nevertheless, these communications are one of the first pieces of historical evidence of bioweapon used in a military context.

During the nineteenth century, scientists like Louis Pasteur, Robert Koch and Alexandre Yersin founded the science of microbiology with the discovery of antisepsis, pasteurization and vaccination as well as the identification of the pathogens that cause anthrax, tuberculosis and plague. While this work certainly improved medical practice, it could also be diverted for hostile purposes.

In World War I, a chemical weapon (mustard gas) was used for the first time in Ypres (France) by the Germans, leading to a defeat of the French. Following this attack, several countries set up national programs to develop chemical and biological weapons for either defensive or offensive applications, despite it being almost impossible to differentiate between the two. A number of State-sponsored program for bioweapon development are known. The Soviet program, called Biopreparat, was a major one. This program started in 1919 and ended in 1991 with a budget estimated at a billion dollars a year [14]. Eight biological agents (smallpox, plague, tularemia, glanders-like, equine encephalitis, anthrax, Q-fever and Marburg virus) were militarized and tons of biological agents were produced. Several biological agents were genetically-engineered to increase their pathogenicity and resistance to existing treatments. The danger of such programs is illustrated by the accident that occurred in Sverdlovsk in 1979 in an anthrax spore production facility for the Ministry of Defense's Scientific Research Institute of Microbiology. After a maintenance operation, some back-up particle filters in the air conditioning system were not replaced and anthrax spores were nebulized through the facilities and spread in the environment, leading to a major outbreak (66 deaths from inhalational anthrax). Following this accident, mean lethal dose (hLD_{50}) for inhalational anthrax in human beings was estimated at between 8000 and 10,000 spores [75, 103]. Another major State-sponsored program was started in Japan in 1925 with about 3000 employees. The main center was based in Manchuria and was called unit 731 (officially "Army Epidemic Prevention Research Laboratory"). Unit 731 produced several tons of pathogens causing cholera,

smallpox, botulism, bubonic plague, anthrax, tularemia and venereal disease. Thousands of Chinese prisoners were exposed to these pathogens and left untreated in order to study the effects of the induced diseases [12, 42, 100]. Some of these pathogens were also disseminated in China, either directly by inoculating reservoirs and wells or indirectly using vectors such as fleas or bombs; it has been estimated that more than 580,000 people may have died [11, 55]. An example of how difficult it is to control bioweapons in a military setting comes from 1942 when bioweapons being used against the Chinese ended up killing more than 1700 Japanese soldiers [134]. Following this event, experiments in nature were stopped and Unit 731 was disbanded after the invasion of Manchuria by the Soviet army. The British program focused on anthrax provides an example of large-scale bioweapon environmental testing. In 1942, *Bacillus anthracis* spores were disseminated in the Gruinard Island using N-bombs. The Island was subsequently quarantined until it was thoroughly decontaminated with formaldehyde in 1990. More recently in Iraq, a program of bioweapon development was initiated in 1985. This program was carried out in factories disguised as chicken-feed plants. Equipment like desiccators and fermenters as well as biological strains were legally bought from Western companies. Specifically, 24 pathogens, including the biological agents of botulism, anthrax, gangrene and brucellosis were purchased from the American Type Culture Center. The program was only stopped in 1991, after the invasion of Iraq by American troops in the Gulf War. In their report, United Nations inspectors estimated that 8500 liters of anthrax, 19,000 liters of botulinum toxin and 2200 liters of aflatoxin had been produced and introduced into explosive systems for dispersion, e.g. 157 R-400 bombs and 25 *Scud* missiles were filled with 3 different biological agents [47, 125, 139].

9.1.3.2 Protocols Restricting the Utilization of Bioweapons

Since the beginning of the twentieth century, the international community has tried to limit the proliferation of bioweapons. The Geneva Protocol of June 14, 1925 was the first protocol that limited the use of bioweapons in war. Two major limitations of this protocol were: although the use of bioweapons was restricted, possessing and developing them was not; and no organization was created to enforce the Protocol.

A convention on the prohibition of the development, production and stockpiling of bacteriological (biological) and toxin weapons and on their destruction (also referred as the "Biological Weapons Convention") was opened for signature on April 10, 1972 and entered into force on March 26, 1975 after ratification by 22 governments [1]. This Convention was the first multilateral disarmament treaty banning an entire category of weapons, as States party to the Convention undertook "*never in any circumstances to develop, produce, stockpile or otherwise acquire or retain microbial or other biological agents, or toxins whatever their origin or method of production, of types and in quantities that have no justification for prophylactic, protective or other peaceful purposes […] weapons, equipment or means of delivery designed to use such agents or toxins for hostile purposes or in armed conflict*.". Today only 155 out of the 171 governments that signed the convention have ratified it. A major limitation is that no penalty was foreseen in case of violation of the convention. To overcome the limitations of this protocol, since 1985, the "Australia Group", an informal group, seeks to ensure that international exports do not contribute to the development of chemical or biological weapons, through the harmonization of export controls among the 41 States-Parties. Coordination of all national export regulations helps Australia Group members to fulfil their obligations under the Biological Weapons Convention to the fullest extent possible. The Australia Group defined a list of biological agents that could be used for the development of bioweapons and for which exports must be regulated [7].

Following the end of the Cold War and the rise of terrorism, the United Nations Security Council unanimously adopted on April 28, 2004 Resolution 1540(2004) which affirms that the proliferation of nuclear, chemical and biological

weapons and their means of delivery constitutes a threat to international peace and security. The resolution obliges *inter alia* States to refrain from supporting, by any means, non-State actors from developing, acquiring, manufacturing, possessing, transporting, transferring or using nuclear, chemical or biological weapons and their delivery systems. On June 29, 2012 the Security Council adopted the resolution 2055(2012), which enlarged the group of experts supporting the work of the 1540 committee to nine experts.

9.1.3.3 Use of Biological Agents in Bioterrorism and Assassination

Currently, bioweapons are unlikely to be used in a military context because of the regulations and for practical reasons, *i.e.* bioweapon development and use is forbidden by the United Nations and it is difficult to control the spread of the biological agent. Specifically, the risk of contaminating your own troops, population and environment is high which is generally unacceptable to an aggressor, even one who has not signed any non-proliferation convention. Currently, the main threat is the use of pathogens by terrorists, that would have major psychological impact in addition to mortality [31].

Several historical instances of proven or suspected use of biological agents for terrorism or assassination have been reported (Table 9.4). Biological agents may be easier for terrorists to obtain than many chemicals or radioactive substances, e.g. ricin—not even classified in CDC Category A—is considered by many as having great potential in bioterrorism. Despite low toxicity compared to other toxins (LD_{50} 1 $mg.kg^{-1}$ compared to 1 $\mu g.mL^{-1}$ for botulinum neurotoxin by ingestion), ricin can be relatively easily purified from the seeds of *Ricinus communis*, a ubiquitous plant (commonly found in parks throughout the world). This toxin has been used for several assassinations such as that of Georgi Markov in London by the KGB using a spiked umbrella that could be used to fire a ricin-containing sphere [87]. In September 2001, letters containing anthrax spores were sent to American government officials. In 2008, the FBI investigations

Table 9.4 Examples of use of biological agents for terrorism and assassination

Date	Examples of utilization of biological agents in the bioterrorist or assassination context	Impact
27th of May 1942	Jan Kubis, a Czech member of the resistance used grenade coated with botulinum toxins to killed the Nazi general Reinhard Heydrich	Success: Reinhard Heydrich seems to died from botulism
1978	KGB killed Georgi Markov in London with a system dissimulated in an umbrella and injecting spheres containing rich	Success: death of Georgi MARKOV
1984	Rajneesh cult contaminated salad bars with Salmonella typhimurium is Dalles (USA)	Success: 751 persons contaminated and 45 hospitalized
April 1990	Aum Shinrikyö sect tried to spray what they thought was botulinum toxinin Tokyo, Yokohama, Yokosuka and Narita with nebulizer placed in trucks	Failed: they didn't succeed to isolate Clostridium botulinum from the soil
June 1993	Aum Shinrikyö sect tried to disseminate anthrax spores from trucks	Failed: nebulizer filter were obstructed
Summer 1993	Aum Shinrikyö sect tried to disseminate anthrax spores in Tokyo from a roof	Failed: utilization of the non-virulent 34F2 strain
Autumn 2001	Letters containing anthrax spore were sent to American officials (probably sent by the researcher Bruce Ivins)	Success: 5 deaths over 11 persons contaminated
April 2013	3 letters containing ricine were sent to the president of the USA and to American officials	Failed: no contamination
2016	DAESH planned to used anthrax in a mall in Nairoby.	Failed: attack thwarted by Kenyan police.

This table lists some examples of reported-uses (proven or suspected) of biological agents for terrorism or assassination [10, 15, 30, 57, 79, 87, 118]

identify Bruce Ivins, a researcher from the Fort Detrick research center, as the main suspect in this attack. Ivins could have stolen spores of *Bacillus anthracis* from the laboratory and, being immunized, he could safely handle the material. This example underlines how easy it is, even for a single man, to develop a bioweapon and use it successfully, if sufficiently competent and determined.

9.2 Antibodies for Biodefense

9.2.1 Antibodies As "Magic Bullets" to Fight Bioweapons

Antibodies are perfect tools to fight bioweapons. Since the end of the nineteenth century, antibodies have been shown to be effective in the treatment of a number of diseases as well as in diagnosis. Although they were supplanted in the twentieth century by antibiotics and other drugs in the treatment of infectious diseases they are still widely used to treat diseases caused by toxins (such as venom). Moreover, multidrug-resistant bacteria have spread naturally and in the context of biowarfare agents, biological agents could have been selected or engineered for their resistance to existing treatments. The development of antibodies is strategic as they can be used when other specific therapies are not available or ineffective and since they can act synergistically with other drugs. One of the main advantages of antibodies compared to other treatment modalities is their specificity of action, which decreases the risk of toxicity and adverse reactions. As antibodies are well-tolerated and their half-life can be as long as 21 days (or even longer if their Fc portion has been appropriately engineered), they are ideal for prophylaxis, e.g. for soldiers who might be exposed to bioweapons on the battlefield. When a possibility of biowarfare is suspected, the medical corps could administer the antibodies before the attack, affording weeks of powerful protection. If contamination with a biowarfare agent is detected in a soldier (military context) or a civilian (bioterrorist context), antibodies could also be administered prophylactically to everyone in the area. Such use is possible because antibodies protect so quickly, unlike other forms of prophylaxis such as vaccination, which generally requires several spaced out doses to elicit full protection. In the context of biodefense, global vaccination is generally not feasible if the biological agent is not encountered in the nature (such as smallpox). In some case, such as botulism, global vaccination is ethically questionable, as it would prevent the therapeutic use of botulinum toxin (which is used to treat more than 25 diseases as well as in plastic surgery).

Modern recombinant antibodies are very safe and can be administrated at high doses to elicit strong protection; this is important because deliberate contamination might involve higher concentrations of pathogen than natural exposure. Nevertheless, there are some limitations to the uses of antibodies. Firstly, if several injections of the same antibody formulation are administered (such as in a context of long-term prophylaxis or prolonged hospitalization), the body can produce its own antibodies against the exogenous antibody (referred as anti-drug antibodies) leading to their neutralization. However, this problem would only apply to prophylactic use, as for therapy a limited number of doses is involved and repeated contamination with the same agent is unlikely. A second limitation is that antibodies have to neutralize the biological agent before it enters its target cell. Considering this limitation, antibodies should be administered as soon as possible after contamination that could be difficult following mass contamination because medical facilities would be saturated and antibody doses might have to be reserved for the medical staff. However, even administered several hours or days after contamination, antibodies could be effective, e.g. following contamination by a virus or a bacterium, antibodies could neutralize any toxins or virions produced *in vivo*. Efforts are also being made to develop transbodies that can pass through the cell membranes and intrabodies that are directly synthesized inside the cell [70, 119]. In the context of biodefense, transbodies would neutralize a biological agent after cell penetration, thereby prolonging the therapeutic win-

dow would be essential in a context of mass contamination. Compared to the production costs of small chemical inhibitors, those of recombinant antibodies are high. However, production costs might be compensated for high success rates during clinical development and the pre-ordering of doses by States for strategic stockpiling would guarantee a fast return for companies developing antibodies for biodefense.

Another advantage of antibodies is that they could also be used for the development of diagnostic tools such as lateral or vertical flow immunoassay on a strip or column. This specific aspects of antibodies will be only presented briefly in this paragraph because it could be the topic of a separate review. Immunoassays are highly specific and sensitive which is ideal for rapid, preliminary detection of a biological agent in different sample types (blood, feces, water, food…). Such tests are generally less sensitive than other methods such as PCR or *in vivo* mortality assay but, as they are portable and fast, they are useful for preliminary screening of potentially contaminated people and samples, even in places where there is no direct access to biomedical or hospital facilities. Moreover, toxins cannot be detected by PCR and the methods necessary such as mass spectrometry are time-consuming, expensive and not always available. Immunodiagnostic tests are generally available for first responder personnel and troops on the battlefield. The Ebola crisis in Africa highlighted the need for easy, rapid and cheap diagnostic tests to detect biological agents in countries where medical facilities are limited. An immunoassay test, called eZYSCREEN developed for Ebola virus takes less than 15 min and just requires a few microliters of blood or serum. In 2014, this was tested in Guinea (Galais et al., Bulletin de la société de pathologie exotique, in press). Other antibody-based diagnostic tools have been developed for a number of biowarfare agents [6, 23, 44, 58, 96] and antibodies for diagnosis—unlike those intended for therapy—do not need to target a neutralizing epitope. In the development of therapeutic antibodies, most of the antibodies isolated specifically interact with the antigen in the (sub-)nanomolar range, but do not neutralize it. Developing immunodiagnostic assays based on such antibodies is a good way of profiting from all the time and money invested.

Considering the advantages of antibodies vis-a-vis managing a biological crisis, several States have drawn up strategies for biowarfare prevention and large-scale treatment. Specifically, some have stockpiled medical countermeasures such as antibodies (in solution or lyophilized). Section 9.2.3 will present monoclonal, polyclonal, oligoclonal and recombinant antibodies currently on the market or in clinical development, directed against the main biowarfare agents. If no such antibody is referred to in the literature, antibodies or antibody fragments at an early stage of development will be presented.

9.2.2 Mechanism of Action of Antibodies for Biodefense

CDC Category A groups together the six biological agents of major concern for biodefense. These are viruses, bacteria and toxins, and all of them could be efficiently neutralized by antibodies acting through similar mechanisms. This paragraph will present briefly the pre- and post- cell-attachment mechanisms neutralization.

Toxins may be the easiest biological agent to neutralize with an antibody. In natural infections, toxins are generally ingested (e.g. food-borne botulism or ricin intoxication), inhaled (e.g. anthrax) or directly produced by the bacteria in the body (e.g. wound botulism). In the context of biodefense, direct production of toxin in the body is unlikely; the toxin would be introduced via the food or water supply chain, or by inhalation. In this case, antibiotics would be ineffective and therapy would be based on supportive therapy or the administration of specific inhibitors, if any are licensed. In this situation, antibodies could rapidly neutralize the toxin's activity *in vivo* (Fig. 9.2). Toxin-dependent toxicity is generally based on two main steps: toxin binding and internalization into the target cell, and then catalytic activity (toxins referred as "A-B toxins"). Antibodies can neutralize such toxins by blocking any of these steps if they target a neutralizing

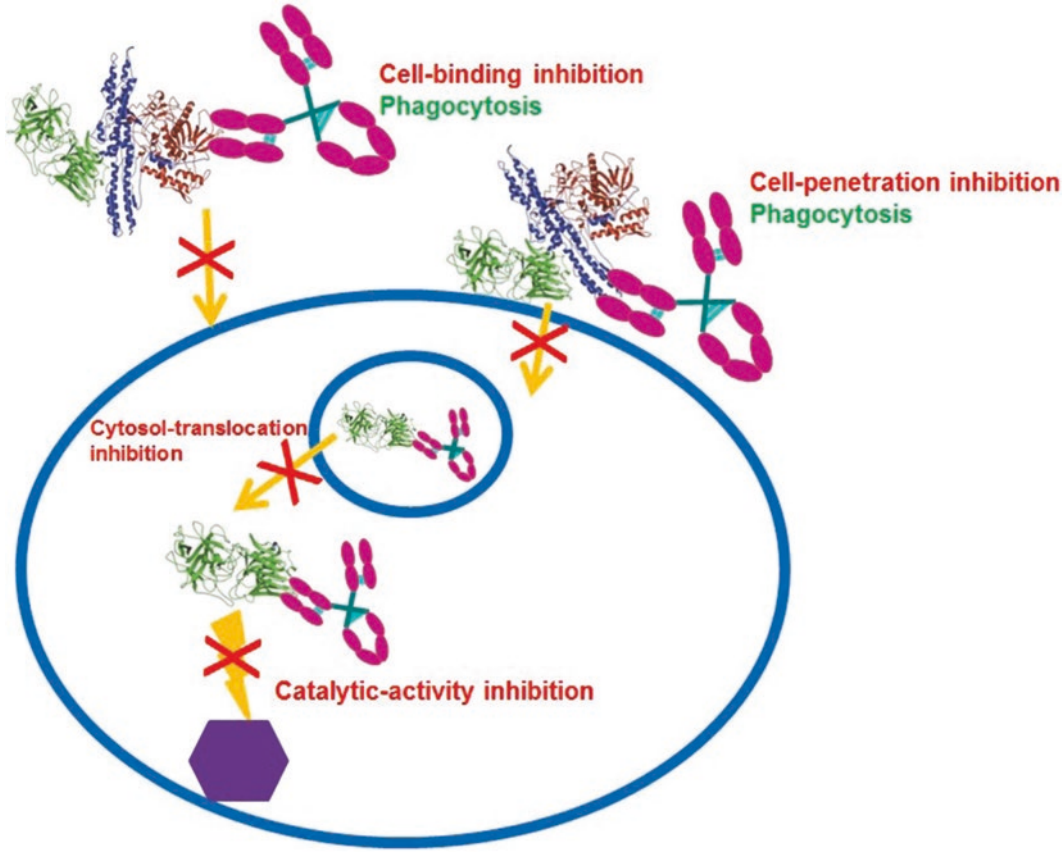

Fig. 9.2 Mechanisms involved in antibody-mediated toxin-neutralization
Proportions between different sizes are not respected in this schematic representation. Toxin actions inhibited are represented in red and antibody effector functions are represented in green

epitope. As toxins may act at very low concentration (e.g. the LD_{50} of botulinum neurotoxin A is 1 $ng.kg^{-1}$), the probability that a therapeutic antibody will bind a toxin in the bloodstream is relatively low. Therefore, it is very unlikely that an antibody will bind two toxins simultaneously (only one of the two binding domains will bind a toxin). Additionally, in contrast to viruses or bacteria, there is no significant repeat motif in toxins and avidity effects will not be involved in toxin-neutralization. Considering these specificities, antibodies must bind to the toxin with a very high affinity (nano- or pico-molar) to preclude dissociation of the antibody-toxin complex. If the antibody epitope is localized in the binding domain of the toxin, the antibody would bind the toxin before it interacts with the cells. If the epitope is based in the internalization domain, the antibody could bind the toxin in the bloodstream or possibly after it has bound to the cell. Antibodies can also bind the translocation domain and inhibit passage of the toxin from endocytic vesicle to cytosol. If the epitope is based on the catalytic domain, the antibody could act indirectly by steric hindrance to prevent binding or internalization, or it could act directly by blocking the toxin's catalytic activity. If the antibody-toxin complex is formed in the general circulation, it will be eliminated by immune cells recruited by the Fc domain of the antibody. A risk is that antibodies could also bind to the toxin on the cell-surface, and induce antibody-dependent cellular toxicity (ADCC) that could finally kill the cell. One limitation of antibodies is that they must be

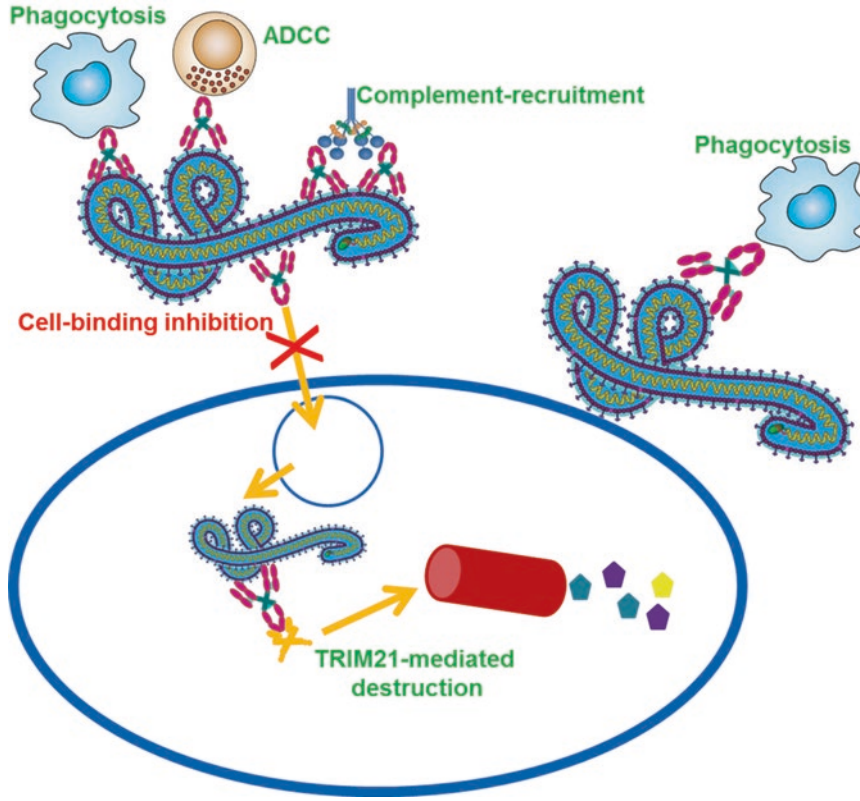

Fig. 9.3 Mechanisms involved in antibody-mediated virus or bacterium neutralization
Antibodies are involved in adcc, phagocytosis, complement recruitment, direct neutralization and trim21 recruitment. Virus/bacteria actions that are inhibited are represented in red and antibody effector functions are represented in green. Proportions between different sizes are not respected in this schematic representation. Ebola schematic representation: Dr. Ian M Mackay, virologydownunder.blogspot.com.au. Yellow: trim21. Red cylinder: proteasome

administered as soon as possible, before toxin-internalization, because unengineered antibodies alone cannot get internalized into the cell to bind its target. Once internalized, an intracellular toxin will persist until it is broken down or the cell dies. In the case of botulism, the toxin does not kill the cell but with the half-life of the botulinum neurotoxin A (BoNT/A) being of the order of months, its effects could persist for a very long time [124]. In the case of contamination with bacteria, antibodies will be able to neutralize any toxin produced *in vivo* in this case antibiotics and antibodies would act synergistically, both neutralizing the activity of the toxin and killing the bacterium.

In the context of bacteria (such as *Francisella tularensis*) or viruses (such as Ebola virus), the main mechanism of neutralization is based on ADCC and phagocytosis (Fig. 9.3) [60]. Viruses and bacteria are larger than toxins and their cell-surface is generally composed of one or more proteins present in multiple copies (often hundreds or thousands of copies) which are usually densely packed together. In this case, different antibodies could target a single virus or bacterium and both antibody binding-domains can bind their target. With such avidity effects, even if one of the two antibody binding-domains dissociates from its epitope, the antibody still remains linked to the biological agent through its second binding-domain and ultimately the free binding domain will be able to rebind. The binding of multiple antibodies on the biological agent's surface will result in efficient recruitment

of complement, phagocytes and cytotoxic cells that will ultimately result in the destruction of the pathogen. Such destruction could be also facilitated by the formation of large immune complexes that diffuse slowly in the organism and that actively recruit the immune-system cells. Nevertheless, fewer cells may end up being infected than by a monodisperse suspension of virions, but those that do may attain a higher multiplicity of infection. If the epitope is directly based on the bacteria/virus cell-binding domain or near this domain (steric hindrance), it could prevent the binding and will lead to the elimination of the pathogen by the immune system. The antibody could also target the pathogen after the binding on the cell surface and indirectly block endocytosis by steric hindrance. Such, antibodies that bind the pathogen post cell-attachment may also act partly by competition with receptors. In this case, higher concentrations of antibodies are generally required because of the valency of the virus-receptor interactions and the strengthening of initial attachment. Although previously thought to mediate protection solely in the extracellular environment, recent research has revealed that antibody-mediated protection also extends to the cytosolic compartment of cells. Indeed, internalized antibody-virion or antibody-bacteria complexes could recruit a cytosolic Fc receptor called TRIM21 (tripartite motif containing 21) that mediates antibody-dependent intracellular neutralization (ADIN) [37, 73]. TRIM21 is a ubiquitously expressed E3 ubiquitin ligase that binds IgG, IgA and IgM with subnanomolar affinity and that directs the pathogen to the proteasome for degradation and that lead to the synthesis and secretion of pro-inflammatory cytokines and chemokines. It is interesting to note that sometime the direct neutralization of virus could be complicated, in part for the neutralization of virus that can be transmitted directly from cell to cell via a virological synapse. In this case, antibodies that interfere with the formation of the synapse by binding to cellular structures and antibodies that counter virion formation or release might block this mode of transfer and indirectly contribute to the neutralization of the pathogen.

9.2.3 Antibodies Against Biowarfare Agents

9.2.3.1 Antibodies Against Anthrax

Anthrax is a disease historically caused by the toxins secreted by the Gram-positive, spore-forming, bacterium *Bacillus anthracis*, but it was recently described that *Bacillus cereus* biovar *anthracis* can also cause anthrax [19]. Distinction is made between four forms of anthrax according to the route of entry: cutaneous, pulmonary, digestive and injection. Pulmonary anthrax (inhalational anthrax) is the main threat for biodefense because spores can be nebulized and the fatality rate is ~100% when left untreated, and up to 88% if treated (45%% following the attacks of 2001 with contaminated letters) [52, 54]. *B. anthracis* contains two plasmids coding for the virulence factors, in particular the toxin subunits. The lethal toxin (LT) is composed of the protective antigen (PA) and the lethal factor (LF); the edema toxin (ET) is composed of PA and the edema factor (EF). Targeting PA and LF with antibodies is a widely-used strategy to stop the pathogenic process. As PA is shared between ET and LT, the majority of the antibodies developed against anthrax target this subunit. New-Zealand rabbits and non-human primates are reported by the US Food and Drug Administration (FDA) as the best model for the evaluation of anti-anthrax antibodies [36, 74]. Following the Sverdlovsk accident, the hLD_{50} was estimated between 8000 and 10,000 spores [75, 103]. Human anthrax prophylaxis is based in the USA on vaccination with BioThrax® (Emergent BioDefense Corporation, Michigan) of the population with high risk of exposure (soldiers, etc.). This vaccine is produced from culture filtrates of a toxigenic, but avirulent, non-encapsulated mutant V770-NP1-R of the *B. anthracis* Vollum strain and is composed chiefly of PA with small amounts of LF and EF that may vary from batch to batch. Post-exposure treatment of inhalation anthrax is based on 60-day course of antibiotics and serotherapy [18]. To complete the pharmacopeia, development programs have mainly focused on developing drugs to prolong the therapeutic window and decrease treatment time; significant efforts have

been made to develop recombinant antibodies against anthrax (Table 9.5).

Raxibacumab (ABthrax®) was the first recombinant antibody to get FDA approval (in 2012) for the prevention and the treatment of inhalational anthrax. This fully human antibody was isolated by phage-display panning of a Cambridge Antibody Technology human antibodies library. Raxibacumab binds PA with an affinity of 2.78 nM [78]. For therapeutic purposes, Raxibacumab should be administered intravenously at 40 $mg.kg^{-1}$ in adults or 80 $mg.kg^{-1}$ in children ≤15 kg, after diphenhydramine premedication. As it is not ethical to contaminate healthy volunteers with anthrax and there are not enough natural cases of inhalational anthrax, the effectiveness of Raxibacumab was assessed in New Zealand white rabbits and cynomolgus macaque models, and safety was assessed in healthy volunteers. Raxibacumab prophylactically administered at 5, 10 or 20 $mg.kg^{-1}$ subcutaneously 2 days prior, or concurrently at 40 $mg.kg^{-1}$ intravenously to rabbits (n = 12/group) challenged with 100LD_{50} aerosolized Ames spores afforded survival rates of 40%, 83%, 83% and 100% respectively, compared to 0% with placebo. The 40 $mg.kg^{-1}$ dose prophylactically administered subcutaneously to non-human primates-NHP- (n = 10) 2 days prior to a challenge with 100 LD_{50} aerosolized Ames spores was 90% protective. Treatment was also evaluated in rabbits and macaques exposed to 200 LD_{50} of aerosolized Ames spores. A 40 $mg.kg^{-1}$ single-bolus of Raxibacumab was administered intravenously to rabbits and to macaques after the detection of PA subunit in the serum or following a 1.1 °C rise in temperature. Raxibacumab post-exposure treatment provided 44% (n = 18) and 64% (n = 14) survival, respectively [78]. The safety of Raxibacumab has been evaluated in three clinical trials, in 326 healthy subjects treated with one or two doses of 40 $mg.kg^{-1}$ of Raxibacumab or of a placebo, alone or in combination with ciprofloxacin. During the clinical studies, only four subjects (1.2%) had their infusion of Raxibacumab discontinued for adverse reactions. Anti-drug antibodies were not detected in any Raxibacumab-treated human subjects during the clinical studies.

Obiltoxaximab Anthim®, is a chimeric (mouse/human) antibody isolated in 1988, but that was further engineered to increase its affinity and to decrease its immunogenicity [124]. After affinity-enhancement, it binds PA with an affinity of 0.33 nM. Obiltoxaximab tolerance-improvement was realized with the DeImmunisation® technology that localized and removed T-cell epitopes through the combined use of immunological and molecular biology techniques. Obiltoxaximab effectiveness was assessed in the rabbit model. A single 10 mg (~4 $mg.kg^{-1}$) dose of Obiltoxaximab administered intravenously 30–45 min prior to an exposure of 163 or 286 LD_{50} aerosolized Ames spores, provided 100 and 88% protection, respectively. For treatment assessment, rabbits were exposed to 172 LD_{50} of aerosolized Ames spores. Twenty-four or 36 h post-infection a single bolus of 10 mg of Obiltoxaximab was administered intravenously and 80% or 50% survival rates were observed, respectively. For therapeutic purposes, Obiltoxaximab should be administered at 16 $mg.kg^{-1}$ in adults or 32 $mg.kg^{-1}$ in children ≤15 kg, by intravenous route, after diphenhydramine premedication. Obiltoxaximab should only be used for prophylaxis when its benefit for prevention of inhalational anthrax outweighs the risk of hypersensitivity and anaphylaxis. The safety of Obiltoxaximab has been evaluated in 3 clinical trials, in 320 healthy subjects treated with one or two doses of 16 $mg.kg^{-1}$ of Obiltoxaximab or of a placebo, alone or in combination with ciprofloxacin. During the clinical studies, only eight subjects (2.5%) had their infusion of Obiltoxaximab discontinued for adverse reactions. Eight subjects who received at least one dose of Obiltoxaximab were positive for anti-drug antibodies, with titers ranging from 1:20 to 1:320, underlining a potential immunogenicity despite deimmunization.

Anthrivig® (anthrax immunoglobulin intravenous, AIGIV) is a polyclonal preparation derived from the plasma of humans immunized with BioThrax® vaccine with a high titer for PA, currently in clinical phase 3 [81]. Anthrivig® effectiveness was assessed in the rabbit and macaque model. For therapeutic evaluation, rabbits

Table 9.5 Anti-anthrax antibodies

INN (International Nonproprietary Name)	Proprietary name	Company	Species	Format	Target	Development Technology	Clinical indication	Administration	Development status	Approval
Raxibacumab	ABthrax®	GlaxoSmithKline (Brentford UK)/ Human Genome Sciences Inc. (HGSI) (Rockville MD USA)	*Homo sapiens*	IgG1 – lambda	PA	Human antibody phage display naïve library	Anthrax inhalation (treatment)	Intravenous	M	FDA: December 14, 2012
Obiltoxaximab	ANTHIM®	Elusys Therapeutics, Inc. (Pine Brook NJ USA)	Chimeric (Mus musculus/ human)	IgG1 – kappa	PA	Hybridoma then deimmunization	Anthrax inhalation (prevention and treatment)	Intravenous	M	FDA: March 18, 2016
	Anthrivig™	Emergent BioSolutions Inc./ BARDA/NIAID	*Homo sapiens*	Polyclonal	PA (at least)	Isolated from the plasma collected from healthy volunteers who had been immunized with BioThrax.	Anthrax inhalation (treatment)	Intravenous	Phase 3	
	Valortim®	PharmAthene/ Bristol-Myers Squibb (formerly Medarex)	*Homo sapiens*	IgG1 – kappa	PA	Transgenic mouse (HuMab mouse)	Anthrax inhalation (prevention and treatment)	Intravenous and intramuscular	Phase 1	
	Thravixa™	Emergent BioSolutions Inc.	*Homo sapiens*	IgG1 – kappa	PA	Isolated from the plasma collected from a healthy volunteer who had been immunized with BioThrax.	Anthrax toxemia (treatment)	Intravenous	Phase 1	

This table list the antibodies that are currently on the market or in clinical trial. Data were compiled from the scientific literature and from the IMGT/mab database [63]

(n = 18/group) were challenged with 204 LD_{50} (±47 LD_{50}) aerosolized Ames spores provided. Anthrivig® was administered at 14.2 or 21.3 mg.kg^{-1} (doses corresponding to anti-PA immunoglobulin) 12 or 24 h post-challenge. Anthrivig® administered 12 h after challenge provided 89% and 100% survival rate, respectively. Twenty-four hours after challenge, Anthrivig® provided 39%% survival, regardless of the dose administered. In the macaque model, animals were challenged with 281 LD_{50} (±51 LD_{50}) and Anthrivig® was administered only after ELISA-detection of PA in the macaque serum. Up to 33%% survival rate was observed, when macaque were treated with an intravenous dose of 21.3 mg.kg^{-1}, compared to 0% in the animal group treated with Gamunex. Regardless of the dose of Anthrivig® administered and the survival outcome, all macaques showed a decrease in circulating PA levels following treatment. The safety and the pharmacokinetic of Anthrivig® were assessed in 125 healthy volunteers, with 3 doses of Anthrivig®. Pharmacokinetic data suggests that a human dose of 7 mg.kg^{-1} is similar to a dose of 21 mg.kg^{-1} in the animal models. No major issue was observed during the safety assay.

Valortim® (MDX-1303), is a fully-human anti-PA IgG1 isolated from transgenic mice, currently in phase 1 [101, 129]. Treatment was evaluated in rabbits and macaques exposed to 200 LD_{50} aerosolized Ames spores. In the rabbit model (n = 10), two intravenous doses of 1 mg.kg^{-1} of Valortim® given 1 h and 3 days post-infection, respectively, provided 90% survival. Later treatment still provided 89% (n = 9) protection, but with two doses of 10 mg.kg^{-1} given twice at 24 h and 120 h post-challenge. Treatment of NHPs (n = 6) demonstrated complete protection after a single intramuscular injection of 1 mg.kg^{-1} Valortim® given 1 h post-challenge. A pharmacokinetic and safety assay was realized in 46 healthy volunteers (43 of them completed the study and 3 stopped it). A single intravenous dose ranging from 0.3 to 20 mg.kg^{-1} or a single intramuscular dose of 100 mg.kg^{-1} was administered. Sixteen volunteers had a grade 1 adverse reaction, but no volunteers had grade 2–4. No volunteers developed anti-drug antibodies [101].

Thravixa® (AVP-21D9), is a fully human IgG1 isolated from humans immunized with BioThrax® by hybridoma technology and immortalized with Epstein Barr Virus lymphocyte. It binds PA with an affinity of 82 pM [105]. Protection and treatment by Thravixa® was evaluated using both New-Zealand white and Dutch Belted Dwarf rabbits, yielding equivalent results. An antibody dose of 10 mg.kg^{-1} administered subcutaneously concurrently with an aerosol or intranasal challenge with 100 LD_{50} of Ames spores (n = 12/group) was fully protective [91, 92]. Regarding post-exposure treatment, subcutaneous administration of Thravixa® (2 mg.kg^{-1}) to rabbits at 0, 24 or 36 h following an aerosol challenge by 102 LD_{50} Ames spores respectively provided 100%, 66% and 33% protection. New-Zealand white rabbits were challenged with an aerosol of ~200 LD_{50} and treated 24–38 h after a significant temperature increase or after PA detection in serum. In rabbits, 92% survival was observed with an intravenous dose of 5 mg.kg^{-1} (versus 0% with a placebo). Similarly, 48 cynomolgus macaques where challenged after PA detection in serum. A 70%% survival-rate was observed with an intravenous dose of 5 mg.kg^{-1} (versus 0% with a placebo). Safety and pharmacokinetic was assessed in 50 healthy volunteers; 40 of them received an intravenous antibody dose of 0.3, 1.3 or 10 mg.kg^{-1} and 10 received a placebo. No severe adverse reaction was observed after infusion [69].

Other antibodies are also in early stage of development. Antibody 35PA83 targeting PA was isolated from a NHP with recombinant PA and humanized to increase its tolerance. It is protective in the New-Zealand white rabbit model and it is in preclinical development with a phase 1 trial due to start soon [89]. Similarly, antibody 2LF protects rabbit from anthrax challenge and it is one of the only isolated antibodies that targets LF and cross-reacts with EF [120]. Such antibodies would be useful if the PA component were naturally or intentionally mutated.

9.2.3.2 Antibodies Against Plague

Plague is a natural infectious disease caused by the gram-negative bacterium *Yersinia pestis*. Distinction is made between three different types of plague namely bubonic, septicemic and pneumonic plague. Bubonic plague is the most common natural type of epidemic plague and it arises when domestic rodent populations and their fleas become infected. Pneumonic plague is of particular interest in biodefense because of its extreme lethality (~100% if untreated), its ability to be transmitted via aerosol and its high infectivity. Pneumonic plague may be contracted by inhaling infectious droplets or develop from untreated bubonic or septicemic plague that spreads to the lungs. Plague is sensitive to antibiotics but they must be administered prophylactically or within 24 h of the onset of the symptoms (which is not always possible in the context of biodefense or a major outbreak). The treatment of choice is streptomycin or gentamicin (doxycycline or ciprofloxacin as an alternative) and supportive therapy. No vaccine or specific inhibitor is yet licensed and strains resistant to antibiotics have been detected [132]. In 1946, killed whole-cell vaccines were developed to protect soldiers and health workers that might be exposed to *Y. pestis* [135]. These vaccines demonstrated protection against bubonic disease but side effects were severe, repeated inoculations were necessary and no protection was observed against pneumonic plague. The live attenuated *Y. pestis* strain EV76, which lacks the pigmentation (pgm) locus required for iron acquisition has been shown to elicit high antibody titers and protect against both bubonic and pneumonic plague when used as a vaccine, albeit with mixed results [88, 121]. *Y. pestis* virulence factors have been identified and represent potential targets for antibodies. Among virulence antigens, F1 is a dominant capsular antigen with antiphagocytic activity, the low-calcium response V antigen (LcrV) is a component of the Type III Secretion System (T3SS) which is essential for pathogenesis, Psa (also referred as Ph6) is involved in resisting phagocytosis and complement-mediated killing, and Survival protein A (SurA) is a chaperone essential for virulence [26, 88, 112]. These proteins are targets of interest for recombinant subunit vaccines and therapeutic antibodies. Evidence of antibody efficacy against *Y. pestis* was provided by two subunit vaccines composed of recombinant F1 and LcrV antigens (one in Phase 2a) that induced an antibody response against both antigens and *in vivo* protection in animal models [26, 136]. However, some studies have shown that some vaccinated NHP succumb to challenge despite possessing high-titer F1/LcrV-specific antibody [110]. It was also demonstrated that passive transfer of specific antibody protects susceptible rodents against pneumonic plague. [110].

The murine monoclonal anti-LcrV antibody mAb 7.3 binds LcrV with an affinity of 80 pM and potently protects mice from death during challenge assays [98]. MAb 7.3 directly neutralizes Yop-dependent cytotoxicity and promotes opsonophagocytosis in macrophages infected with *Y. pestis in vitro* [53]. All mice (n = 10) immunized intraperitoneally with a dose of 35 μg of mAb 7.3 4 h before an aerosol challenge with 88 LD_{50} of *Y. pestis* strain GB, survived. In therapy, survival rates of 80%% and 60%% were observed when mAb 7.3 was administered 24 and 48 h after the challenge, respectively [48]. This protection was described as TNFα and IFNγ-dependent [65].

The m252 anti-F1 antibody, was isolated from a naive human phage-displayed Fab library and expressed as full-length IgG [137]. Despite its isolation from a naïve library, m252 binds F1 with a sub-nanomolar affinity. Protection assays were realized in the mice bubonic plague model. A dose of 500 μg of m252 administered intraperitoneally 24 h before a subcutaneous challenge with ~25–40 LD_{50} of *Y. pestis* CO92 strain increased the mean time-to death from 7 to 13 days and one out of 6 mice survived. When this antibody was administered prophylactically concurrently with two other anti-LcrV antibodies (500 μg each), the mean time-to death increased from 7 to 14 days and 5 out of 6 mice survived, emphasizing a synergistic effect of the oligoclonal mixture. Interestingly, a single administration of m252 48 h post infection provides 100%% survival. These antibodies were not characterized in a pneumonic plague model.

Considering biothreat and the natural cases of plague, the development of anti-plague antibodies is a priority. Such antibodies will be useful for prophylaxis and the specific treatment of plague.

9.2.3.3 Antibodies Against Tularemia

Francisella tularensis, the causative agent of tularemia (also known as rabbit fever and deer fly fever), is a gram-negative, intracellular, non-mobile, coccobacillus, naturally found in the Northern hemisphere. Natural human contamination occurs after skin contact with tissues or body fluids of infected animals of from bites of infected arthropods. In the biodefense context, infection could also be transmitted by aerosol or ingestion of contaminated-food or water. Type A tularemia is caused by *F. tularensis* subspecies *tularensis* and it is the form that is the most virulent and the most likely to be weaponized. The infective dose in humans is extremely low: 10 bacteria when injected subcutaneously and 25 when given as an aerosol [85]. The live vaccine strain (LVS) was developed from *F. tularensis* subspecies *holartica*, which causes Type B tularemia.

There is no consensus on which animal model should be used to evaluate tularemia treatment [85]. Historically, grivet monkeys infected with the SCHU S4 type A strain have been used to study the pathogenesis of tularemia and evaluate vaccines. Rhesus monkeys were also used for aerosol challenges with a strain of the subspecies *holarctica*. Mice have generally been used as an experimental model of tularemia for infection with LVS vaccine or with SCHU S4 strain. It was suggested that mice infected with SCHU S4 strain should be capable of satisfying the FDA animal rule [28]. Recent studies suggest that rabbits are a good model of human pneumonic tularemia when exposed to aerosols containing a virulent, type A strain, SCHU S4 [114]. A two-week course of doxycycline or ciprofloxacin should be effective in prophylaxis and early administration of streptomycin or gentamicin is very effective in therapy. No natural resistance in *F. tularensis* to antibiotics used for clinical therapy has been demonstrated for aminoglycosides, tetracyclines, chloramphenicol and quinolones, but erythromycin resistance is prevalent in Europe. New antibiotics such as ketolides, glycylcyclines, linezolid and new fluoroquinolones are currently under evaluation for therapeutic purposes [17]. Streptomycin- and tetracycline-resistant strains of *F. tularensis* were developed for well-intentioned experimental purposes, but such works could be also a proof of concept for bioterrorists [17, 85]. The LVS vaccine is available as an Investigational New Drug (IND, clinical development phase 2). A one-dose should be administered by scarification, but it was recently shown that LVS not elicit complete protection against lethal challenge with a virulent type A *Francisella* strain [32, 106, 116]. This vaccine fully protects mice against an intradermal but not aerosol challenge with 1000 LD_{50} of SCHU S4 strain. The absence of protection against aerosol contamination is a breach in biodefense. A recent study showed that respiratory and oral vaccination improves protection conferred by the LVS against pneumonic tularemia in the rabbit model, compared to the protection conferred by scarification [114].

Currently, there is no antibody on the market or in clinical development but efficacy has been demonstrated [109]. One explanation could be that the Schu S4 strain completely abolishes the inflammatory responses that are required for efficient antibody-mediated bacterial clearance [59]. However, evidence of antibody efficiency in prophylaxis and treatment has been obtained with the passive administration of immune serum. The administration of immune serum 24 h post infection and every 3 days thereafter protected 90%% of the mice against an intranasal challenge with LVS [59]. First, immune-serum administration 48 h post-infection protected 25%% of the mice and increased the mean time-to death from 9.5 to 13.5 days. A study showed that passive transfer of antibodies directed against the membrane protein fraction (MFP) of *F. tularensis* Schu S4 to mice infected with the same strain resulted in complete protection when combined with gentamicin treatment [117].

Ab63 is a monoclonal IgG3(κ) obtained after the immunization of mice with LVS, with an affinity of 0.75 nM for the *O*-antigen of *F. tularensis* lipopolysaccharide LPS [66].

Administration of an intraperitoneal dose of 200 μg of Ab63 2 h after an intranasal challenge with 24 CFU of Schu S4 did not protect mice from death but slightly increased the mean time-to death.

Antibodies can completely protect against LVS (type B strain) but not against the more virulent Schu S4 (type A) strain, possibly due to different virulence mechanisms between strains. Developing antibodies that neutralize type A and type B strains before naturally antibiotic-resistant strains emerge is a priority for the prophylaxis and treatment of tularemia.

9.2.3.4 Antibodies Against Smallpox

Smallpox is caused by at least two species of *Variola virus*: *Variola major* which causes 10–30%% mortality (95% if untreated) and *Variola minor* that cause less than 1%% mortality. In 1958, the World Health Organization started a global vaccination campaign because humans are the only reservoir of the *Variola virus*; smallpox was officially declared eradicated on May 8, 1980. All vaccines are based on *Vaccinia virus*, another orthopoxvirus that induce a cross-protection against *Variola virus*; *Vaccinia virus* and *Variola virus* share 150 open reading frames and have an overall identity of 90%%. Several live strains ($VACV_{NYCBH}$, EM-63 and Lister) were used for the vaccination but these can induce adverse reactions (~250 cases for 1000,000 vaccinations) that can be fatal (60 deaths for 100,000,000 vaccinations) and historical vaccine production methods are incompatible with current quality rules for medicinal products [104]. In a context where smallpox is no longer found in nature, the balance between the benefits and risks of vaccination is against continued mass immunization. Vaccination was wound down in the years following eradication, and now only those at a high risk of exposure were vaccinated with a second-generation vaccine (such as ACAM2000®, FDA-approved in 2007) although strategic stockpiles of vaccine were accumulated [32, 80]. Following eradication, *Variola virus* stocks were destroyed, and now only two stocks are being kept in BSL4-secure facilities (in Atlanta, USA and in Koltsovo, Russia) for research purposes. However, in the context of the Cold War, we cannot know that all *Variola virus* stocks were indeed destroyed and natural resurgence of the virus may be possible, particularly due to the ice melting in countries were corpses were buried. Currently, only a few individuals are immunized, and the immunological status of people that were vaccinated is undetermined. A study on 680 patients with smallpox estimated that the mortality rate was 52% among those who had never been vaccinated, 1.7% among those who had been vaccinated within 10 years, and 11% among those who had been vaccinated more than 20 years ago [38, 68]. Considering the vulnerability of the population, the high aerosol infectivity (a single virion can cause smallpox) and the relative ease of large-scale production, smallpox is still considered as a major potential biowarfare agent. In the event of a smallpox outbreak in the USA, the CDC would immediately implement the Smallpox Response Plan. As there is currently no FDA-approved specific and effective treatment for smallpox, the plan largely seeks to contain the outbreak. Several bioterrorism scenarios were considered by the CDC. Depending on how many people get infected at the outset, the total number of people contaminated before final containment could vary from 4.200 (10–100 people initially contaminated) to several millions (thousands of people initially contaminated). In this last case smallpox will become endemic and several years would be required to bring the disease under control [43].

Three compounds (cidofovir, ST-246® and CMX-001®) that inhibit smallpox virus replication *in vitro* and in animal models have IND status for emergency smallpox-treatment, but none is yet FDA-approved (clinical trial phase II or phase III against other virus) [16, 84].

The efficacy of antibodies for the prophylaxis and treatment of smallpox has been demonstrated in the past. During infection, the virus exists in two antigenically distinct forms, namely intracellular mature virions (IMV) that are involved in person to person contamination, and extracellular enveloped virions (EMV) that are involved in cell to cell contamination. The membrane pro-

teins of each virion are potential antibody targets and ideally both types of virion would be targeted at the same time because a single antibody may be not sufficient to induce full protection [72]. The role of complement in antibody-dependent virus elimination has been demonstrated [13, 40, 71]. The Vaccinia Immune Globulin (VIG) is a human-derived polyclonal mixture that is approved for the specific treatment of smallpox and of complications resulting from vaccination. Studies on rhesus macaques demonstrated the prophylactic efficacy of VIG against a challenge with monkeypox [34, 72]. Unfortunately, this product is of human-origin and only available in very limited quantities in the USA. Moreover its efficacy is uncertain due to interbatch variability in potency and a lack of understanding of the molecular determinants of protection. In 1941 a study demonstrated that prophylactic administration of serum from convalescents is fully protective ([34, 72]).

Several monoclonal antibodies that cross-neutralize different orthopoxvirus (vaccine, smallpox, cowpox and monkeypox) were isolated by hybridoma technology from peripheral blood mononuclear cells (PBMCs) from a donor who had recovered from a naturally occurring monkeypox virus infection or from otherwise healthy subjects previously immunized with one of three different vaccine formulations [40]. After an initial screening against recombinant antigens (A21, A27, A28, A33, B5, D8, F9, J5, H2, H3, L1, L5, A33 and B5) or inactivated-virus lysate (monkeypox, cowpox and vaccine), 89 hybridomas were identified as reactive. 66% of the monoclonal antibodies that interact with a vaccine-antigen also interact with the orthologue *Variola*-antigen. 54%% of the hybridomas secreted antibodies that neutralized at least one orthopoxvirus (25%% neutralized simultaneously the vaccine, cowpox and monkeypox), with a maximum of neutralization effect (E_{max} > 50%%) at 100 µg.mL^{-1} or lower. IC_{50} values of individual mAbs ranged from ~0.02 to 100 µg.mL^{-1}, and E_{max} values varied from 50% (the designated cut-off threshold to identify potent neutralizing clones) to 99.5%. Interestingly, all anti-B5 antibodies failed to cross-neutralize monkeypox despite anti-B5 antibodies are the main antibodies involved in vaccine neutralization. A mixture of antibodies, containing diverse specificities with high neutralizing (low IC_{50} and high E_{max} values) and cross-neutralizing activities to both IMV and EEV, was designed. MIX4 contained single neutralizing monoclonal antibodies directed to A27, L1, B5, and A33. C57BL/6 mice were intranasally challenged with 10^5 PFU of VACV-WR. One day before, concurrently, or 1, 2 or 3 days after challenge, mice received a single dose of Mix4. Administration of Mix4 up to 1 day post-infection completely protected mice from death and weight-loss. Administration 2 or 3 days after challenge completely protected mice from death but failed to protect mice from weight-loss. Mix4 induced a better protection compared to VIG.

Recombinant and monoclonal antibodies directed against different orthopoxvirus-antigen (such as B5, A33, A27 or L1) were also previously isolated [13, 24, 25, 56, 111]. Many of the antibodies isolated were only characterized against vaccine, monkeypox or cowpox, but only a few of them were characterized against smallpox.

9.2.3.5 Antibodies Against Viral Hemorrhagic Fever

Viral hemorrhagic fevers (VHF) are a heterogeneous group that causes illnesses characterized by fever and bleeding diathesis. They are caused by four virus families: *Filoviridae*, *Arenaviridae*, *Flaviviridae* and *Bunyaviridae*, but this section will focus on *Filoviridae*. The *Filoviridae* include the Ebola and Marburg viruses. The *Arenaviridae* include the etiologic agents of Lassa, Junin, Machupo and Sabia hemorrhagic fevers. The *Flaviviridae* include dengue, yellow fever and two viruses in the tick-borne encephalitis group that cause VHF: Omsk VHF and Kyasanur Forest disease virus. The *Bunyaviridae* include the members of the *Hantavirus* genus that cause hemorrhagic fever with renal syndrome, the Congo-Crimean VHF from the *Nairovirus* genus, the Rift Valley Fever virus from the *Phlebovirus* genus. Many VHF agents are highly infectious, can be nebulized, and their fatality rate can be greater than 90%% in the absence of early, effec-

tive treatment [86]. Specifically, Ebola, Marburg, Lassa and Machupo viruses are the main threat with respect to both biodefense and natural outbreaks. Following its emergence in December 2013, the recent Zaire Ebola virus (EBOV) outbreak in West Africa has spread and persisted for more than 2 years and killed more than 11,000 individuals, making it the largest EBOV epidemic in both scale and geographical extent to date. During this outbreak, the USA, Spain, Norway and France for the first-time decided to repatriate any of their medical personnel who contracted the infection in Africa in order to provide them efficient treatment in secure, domestic medical facilities. Notwithstanding the precautions taken during repatriation and treatment, media coverage contributed to panic in the populations of the countries that repatriated health care workers [45, 51, 107]. This Ebola epidemic was useful with respect to evaluating the ability of Western countries to contain an epidemic by means of specific restriction measures such as the medical checking of passengers coming from West Africa at airports. It was also an opportunity for France to evaluate its capacity to deploy a military hospital directly in Guinea to treat infected health care workers. The outbreak furthermore allowed several countries to test their reaction capabilities that could be similarly deployed in response to a bioterrorist attack involving VHF or other infectious biowarfare agents. Finally, this outbreak afforded an opportunity to test antiviral-drugs and antibodies in clinical development. The development of anti-VHF drugs is complicated by the fact that many of these viruses have to be manipulated in a BSL4 facility.

A consensus on the appropriate animal models of diseases caused by VHF has not yet been reached due to symptom discrepancies between models and humans. Rhesus and cynomolgus macaques are representative models of filovirus infection as they exhibit remarkably similar symptoms to those observed in humans. However, practical and ethical problems limit the experimental use of non-human primate models. Furthermore, there are no inbred, genetically manipulated strains of NHP. Rodent models such as mouse, guinea pig, and hamster, have also been developed but these require adaptation of the virus to produce lethal disease and do not mirror all symptoms of human filovirus infection [35, 83].

In the case of Ebola, several antiviral drugs have been isolated but only three of them (AVI-6002, AVI 7537 and TKM 130803) completed clinical phase 1 and they are specific to the Ebola Zaire strain [21].

Serum from convalescents has been administered with some success to several patients, but only a few results have been published [27, 126]. In a nonrandomized, comparative study, 99 patients from Guinea with confirmed Ebola infection received two consecutive transfusions of 200–250 ml of ABO-compatible convalescent plasma, with each unit of plasma obtained from a separate convalescent donor. The transfusions were initiated on the day of diagnosis or up to 2 days later. No serious adverse reactions associated with the use of convalescent plasma were observed. When compared with 418 patients who had been treated at the same center during the previous 5 months, no significant improvement in survival was observed in the group that was given exogenous serum [126].

ZMapp™ is an oligoclonal mixture composed of monoclonal antibodies, that is currently under clinical phase 2 and that received the orphan drug designation by the FDA on August 25, 2014. It was developed by Mapp Biopharmaceutical Inc., LeafBio Inc. and Dreyfus Inc., starting from two mixtures composed of three monoclonal antibodies each: MB-003 that was developed by the U.S. Army Medical Research Institute of Infectious Disease (USAMRIID) and that is composed of three humanized or human-mouse chimeric antibodies (c13C6, h13F6 and c6D8) and ZMAb that was developed by the Public Health Agency of Canada's National Microbiology (NML) and that is composed of three murine monoclonal antibodies (m1H3, m2G4 and m4G7). ZMapp™ formulation is composed of c13C6 and from c2G4 and c4G7 that were chimerized (human-mouse) [95]. ZMApp™ antibodies binds to Ebola virus glycoprotein ectodomain [122]. A three dose adminis-

tration of ZMapp™ (50 mg.kg^{-1}) up to 5 days post-infection fully-protected non-human primates (n = 6) intramuscularly challenged with 628 PFU of EBOV-K (vs. 0% survival for the animal that received an irrelevant antibody). *In vitro* ZMapp™ cross-neutralized EBOV-G. ZMapp was evaluated in humans in Guinea in 2015 in a randomized study [94]. ZMapp was administered simultaneously to the standard treatment used in Guinea (that specifically includes oral favipiravir) and results were compared to those in a group of patients only administered with the standard treatment. Thirteen of the 35 patients who received the standard treatment died (37%) compared with only 8 of the 36 patients that additionally received the ZMapp (22%). Although the estimated effect of ZMapp appeared to be beneficial, the result did not meet the pre-specified statistical threshold for efficacy. The observed posterior probability that ZMapp plus the current standard of care was superior to the current standard of care alone was 91.2%, falling short of the pre-specified threshold of 97.5%.

Development of pan-Ebolavirus therapy is a priority for the public health. However, development of such antibodies is complex due to antigenic differences between the five ebolavirus species. 6D6 is a mouse monoclonal antibody isolated by hybridoma technology after the immunization of mice with EBOV virus-like particles. BALB/c mice were intraperitoneally challenged with 1000 PFU of mouse-adapted EBOV and 100 µg of 6D6 was intraperitoneally administered 24 h post-infection [39]. The treated animals survived without clinical symptoms, whereas untreated mice succumbed to infection within 9 days. Treatment with 6D6 24 h after infection delayed the onset of the disease caused by these ebolaviruses and significantly reduced the weight loss in this immunocompromised mouse strain. All 6D6-treated mice survived the EBOV infection. C57BL/6 mice were challenged wild-type EBOV and SUDV. Administration of 6D6 24 h post-infection, fully protected mice from death, delayed the onset of the disease caused by these ebolaviruses and significantly reduced the weight loss. Epitope-mapping study revealed that 6D6 targets an epitope that is shared between all known ebolavirus species. More precisely, the epitope was localized in the IFL region of the GP2. These results revealed that 6D6 represents a potential candidate for drug development and that antibodies targeting the same epitopic region should cross-neutralize Ebola viruses.

Following the last Ebola epidemic, efforts were made to develop anti-Ebola drugs. However, efforts should also be made for the development of drugs against the other VHF agents that are also of major concern in public health and biodefense. Antibodies against other VHF have already been isolated but none of them are in clinical development and they are not reviewed here.

9.2.3.6 Antibodies Against Botulinum Neurotoxins

Botulism is a rare, life-threatening disease caused by botulinum neurotoxins (BoNT), secreted by the spore-forming bacterium, *Clostridium botulinum*. Seven BoNTs serotypes (A to G) have been described but serotypes A, B and E are responsible for most cases of natural human poisoning [61]. Between each serotype, amino acid sequences share between 34% and 64%% identity, and subtypes have been identified [49, 61]. Botulinum neurotoxin A (BoNT/A) is the most toxic substance known with a human 50%% lethal dose (LD_{50}) estimated at 1 ng.kg^{-1} (intravenous and subcutaneous routes), 10 ng.kg^{-1} (pulmonary route) or 1 µg.mL^{-1} (oral route) [4, 41, 61]. BoNTs are type A-B heterodimeric molecules composed of a 100 kDa heavy chain (HC) and a 50 kDa light chain (LC). The heavy chain is successively implicated in toxin binding on motor neurons, internalization by dual-receptor-mediated endocytosis and translocation of the light chain into the cytosol. The light chain is a zinc endopeptidase which cleaves a protein of the complex implicated in the exocytosis of acetylcholine. Both chains are potential targets for antibodies. Toxin activity induces flaccid muscular paralysis that progressively spreads to involve all muscles, leading to respiratory distress and eventually death in absence of treatment. Three forms of naturally occurring botulism are described:

foodborne, intestinal and wound botulism. However, BoNTs could also be nebulized and produced a clinical picture very similar to food poisoning.

No specific chemical inhibitor of BoNTs activity is available on the market. A pentavalent vaccine against botulism neurotoxins A to E has been developed and granted IND status but this vaccine is only given to individuals at high risk of exposure. In fact, BoNTs have been introduced as a safe and effective treatment for a wide range of disorders associated with involuntary muscle contractions and spasm disorders, and these ever-increasing medical indications preclude mass immunization against botulism [3, 131].

Infant botulism cases are treated by injections of a human-derived botulinum immunoglobulin preparation (BabyBIG®, approved by the FDA in December 9, 2003), which is well tolerated but only available in very limited quantities and very expensive [5]. Only 31% of BabyBIG®-treated patients experienced a treatment-emergent adverse reaction compared with 45% of placebo-treated patients, and there was no evidence of anaphylaxis in any patient enrolled in the study [108]. Efficacy was evaluated in 122 patients with laboratory-confirmed infant botulism (caused by BoNT/A or B). Fifty-nine received BabyBIG® and 63 received placebo. When administered within 3 days of hospital admission, treatment with BabyBIG® shortened the mean hospital stay of all infant botulism patients by 3.1 weeks (from 5.7 to 2.6 weeks), the length of intensive care unit stay, the duration of mechanical ventilation and the duration of tube feeding.

A heptavalent (A to G) horse-derived derived serum (HBAT®, Emergent Biosolutions) is the only treatment available in USA (FDA approval, March 22, 2013) for both adult and infant botulism. This serum is composed of more than 90%% of Fab and $F(ab')_2$ and of less than 2%% full-length IgG. Despite lot-to-lot variation each single-use vial contains a minimum potency of 4500 Units (U) for BoNT/A, 3300 U for BoNT/B, 3000 U for BoNT/C, 600 U for BoNT/D, 5100 U for BoNT/E, 3000 U for BoNT/F and 600 U for BoNT/G [46]. This serum was already successfully administered in therapy to a patient with foodborne-botulism, and HBAT is under phase 4 to determine its pharmacokinetics in pediatric patients (NCT02051062) [50].

A trivalent horse-derived serum neutralizing BoNT A, B and E (GlaxoSmithKline/Novartis), a bivalent horse-derived serum neutralizing BoNT/A and B and a monovalent horse-derived serum neutralizing BoNT/E (Instituto Butantan) are also available. Both products were no longer provided by the CDC and were replaced by the HBAT® [29].

Nevertheless, due to their animal origin, these equine-derived antitoxins may be poorly tolerated and could induce serious adverse reactions, like serum sickness or anaphylactic shock. Development of human or human-like antibodies is thus required for safe and effective treatment of botulism. 3D12 is a fully human antibody, isolated from humans immunized with pentavalent botulinum toxoid. It binds BoNT/A with an affinity of 32 nM. Intraperitoneal injection of mice with $20LD_{50}$ of BoNT/A (Hall strain) pre-incubated with 50 μG of 3D12 significantly delayed mean time-to death but failed to protect mice from death. However, when a second mouse-derived antibody (C25 or S25) is injected simultaneously (25 μg of each antibody), all mice survived a challenge with 100 LD_{50}. When 3D12 is injected with C25 and S25 (16.7 μg each), all mice survived to a challenge with 5000 LD_{50} and 90%% of mice survived to a challenge with 20,000 LD_{50}. C25 and S25 monoclonal antibodies were humanized to decrease their immunogenicity and engineered to increase their affinity. These three antibodies constitute an oligoclonal mixture for efficient BoNT/A-neutralization. AntiBotABE European framework project developed a combination of five humanized recombinant-antibodies of NHP origin that protect mice from challenge with BoNT/A, B or E [8, 33, 76, 77, 97]. SEM120-IIIC1 and A1HC38 target BoNT/A light and heavy chain, respectively, BLC3 and B2.7 target BoNT/B light and heavy chain, respectively, and ELC18 targets BoNT/E heavy chain. Despite the proximity of NHP-antibodies with human antibodies the five antibodies developed were germline humanized

[9]. All antibodies either administrated alone or in pairs fully protected mice from an intraperitoneal challenge with the toxin of the relevant serotype.

9.2.3.7 Antibodies Against CDC Category B Agents: The Example of Ricin

CDC category A groups together the six agents considered as a priority for biodefense but other systems point up other biological agents as priorities. Some of them may be less fatal but they are nevertheless capable of incapacitating troops and populations, e.g. Staphylococcal Enterotoxin B (SEB) causes symptoms even when inhaled at very low doses in humans. A dose at least 100 times less than the lethal dose by the inhaled route would be sufficient to incapacitate 50%% of those exposed for 1–2 weeks [32]. Ricin is another example of a CDC category B agent that could be used as a bioweapon. Although its LD_{50} is higher than that of other toxins, it is easy to extract from Ricinus communis seeds.

Ricin is composed of a B-subunit (RTB) that binds sugars on the cell surface for cytosolic entry of an A-subunit (RTA) that inhibits the ribosome-dependent protein synthesis. It is lethal by the oral route, but 1000-fold more potent by the pulmonary and parenteral routes. Ricin has an aerosolized LD_{50} of 3–5 $\mu g.kg^{-1}$ in the mouse and 5.8–15 $\mu g.kg^{-1}$ in non-human primate models. There is no licensed prophylaxis or specific chemical compound that neutralizes ricin; the only treatment is supportive therapy. Two recombinant vaccines based on immunization with the RTA subunit are currently in phase 1 and 1 B development, namely RVEc™ (USAMRIID) and RiVax® (University of Texas Southwestern Medical Center) [93, 128, 130].

A proof of concept study demonstrated the efficacy of polyclonal antibodies for the full protection of mice exposed to an aerosol of ricin, when the antibodies are administered 20 min post-exposure. 43RCA is a recombinant antibody that binds ricin with an affinity of 40 pM. This antibody was isolated by phage-display technology starting from a NHP (*Macaca fascicularis*) immunized with the non-toxic A-chain of ricin. This scFv was then germline-humanized to decrease its tolerance. In a cell-free neutralizing-assay, 43RCA neutralized 89% of ricin activity at 40 $\mu g.mL^{-1}$, and 50% at 1.5 $\mu g.mL^{-1}$ (corresponding to a molar ratio [43RCA]/[ricin] of 12) [90]. This antibody was also protective in vivo (unpublished data) and was used for the development of an antibody-nebulizer for rapid and easy self- administration [99].

PB10 is a murin monoclonal IgG2b that binds a linear epitope on the enzymatic RTA chain and that neutralized ricin by interfering with the transport to the trans-Golgi complex net- work, presumably by shunting ricin to lysosomes for degradation [138]. PB10 was chimerized (cPB10) by grafting its variables domains on human IgG1 and K constant domains [115]. Despite an affinity of only 40 nM the IC_{50} of cPB10 is 0.03 $\mu g.mL^{-1}$. When administrated by i.p. 24 h before an i.p. challenge with 10 LD_{50} of ricin, a single dose of 5 µg of cPB10 fully protected mice from death. More interestingly for biodefense, this antibody fully-protect BALB/c mouse from death whatever a single dose of 10 $mg.kg^{-1}$ of cPB10 is administrated 24 h before, simultaneously or 4 h after an aerosol challenge with ~5 LD_{50} of ricin. cPB10 was then germline-humanized (hPB10) to increase its potential tolerance. After humanization, the PB10 variable domains overall identity increase from 70% to >90%, without affecting antibody functions [127]. After humanization, an i.p. administration of 5 or 40 µg of hPB10 24 h before a ricine challenge, fully protected mice from death after an i.p. or an intranasal challenge, respectively, with 10 LD_{50} of ricin. This antibody represents a potential lead drug for the biodefense.

Ten other antibodies were also isolated by phage-display technology starting from 2 non-human primates immunized with ricin-holotoxin or a subunit-vaccine. These antibodies bind ricin with (sub-)nanomolar affinities (5 of them had affinities bellow 1 pM) and neutralized toxin activity in a HeLa Ub-FL cell-based assay. Mice were challenged intranasally with 2 LD_{50} of ricin 6 h before an intravenous administration of 100 µg of antibody. The antibodies MH36, MH75 and MH73 induced significant protection, and

both MH1 and MH77 fully protected mice from death.

9.2.4 Outlook for the Development of Antibody for Biodefense

As reviewed above, considerable advances were done since the 2001 anthrax-crisis, for the development of antibodies against biowarfare agents, but, there are several limitations. First, the majority of the antibodies that were developed target CDCA-category agents. Despites efforts made against A-category agents, antibodies against pathogens such as *Francisella tularensis*, were not yet available. In the context of tularemia, one major issue is that while mice are extremely susceptible to tularemia, rats, rabbits, and non-human primates are relatively more resistant to infection. Perhaps most importantly, emerging evidence indicates that immunity against virulent strains of *F. tularensis* that cause disease in humans differs from protective immune responses elicited by model strains of *Francisella* [109]. A limitation to the CDC A-category is that even these biological agents are considered as the main threat for biodefense the list is not often actualized. As a consequence, efforts should be done for the development of antibodies neutralizing biological agents currently classified among the CDC B-category and CDC C-category (emerging pathogens). Indeed, pathogens of the B-category, such as *Vibrio cholerae*, the Epsilon toxin of *Clostridium perfringens*, some strains of *Salmonella* sp. or *Escherichia* sp., may be used to contaminate the food and water supply chain or the cattle to destabilize a country. CDC B-category agents, such as Enterohemorrhagic *E. coli* (EHEC) may be less lethal than A-agents, but may incapacitate many people for several days or weeks. Such incapacitation will lead to a significant destabilization of the economy or of impacted-regiments. One limitation for the development of such antibodies may be the antigenic diversity among some pathogen families which require identifying carefully the antigen that should be used for the antibody generation. The recent development of bi- or multi-specific antibody would represent a hope to overcome the difficulties related to the antigenic diversity. Indeed, with such antibodies it would be possible to target several serotypes or subtypes of biological agents with a single antibody. The clinical development of such multivalent molecule would also be cheaper than the simultaneous development of several antibodies. Indeed, the clinical development of oligoclonal antibodies requires the assessment of each antibody separately (as a part of the mixture) and the oligoclonal mixture in parallel, which is expensive. The lake of funds is the main explanation for the gap generally observed between research and (clinical-) development, emphasizing the interest for the development of engineered-antibodies. As there is only few or no natural diseases caused each year by the biowarfare agents, the pharmaceutical industry generally invest no money for the development of therapies against such diseases, and Stated should fund such development by their own. Considering the funds required, the possibility of drug-development is restricted to only some countries in the World (Table 9.6). Among these richest countries, some of them are not necessarily directly impacted by terrorism and invest no money for such development. Generally, for national security reasons, States preferred to be self-sufficient and thus to autonomously developed their (medical-) countermeasures. Nevertheless, in a geopolitical context where terrorism affects macro-regions of the World and considering the fact that pathogens can cross easily the borders, the development of medical therapies against biowarfare agents could be done in the context of multi-state collaborations (European Union, North Atlantic Treaty Organization…). For example, the European Framework projects funds research programs in strategic topics, but in the context of therapies, nor the European Framework projects nor European agency such as the European Defense Agency can pay for the clinical development. A key for a "win-win" partnership between States and pharmaceutical industry may be the funding of clinical development by pharmaceutical industries and the commitment of States to stockpile drug-doses. Considering the possibility of fast

Table 9.6 Plan and cost of (pre-) clinical development

	Preclinical development		Clinical development				
	Before market authorization						Post market
	In vitro studies	Animal *in vivo* studies	Phase I safety and dosage	Phase II efficacy and side effects		Phase III efficacy and monitoring of adverse reactions	Phase IV post-market safety and efficacy
Number of human volunteers	0	0	20–100 healthy volunteers or people with the disease/ condition.	Up to several hundred people with the disease/ condition.		300–3000 volunteers who have the disease or condition	All sick-peoples that received the drug.
Success rate	*Highly target-dependent*	*Highly target-dependent*	**70%**	**33%**		**25–30%**	**N.A.**
Primary goal	Looking for leads. *In vitro* confirmation of the efficacy of the molecule.	*In vivo* confirmation of the *in vitro* efficacy. Determination of the optimum dose at which the drug shows maximal biological activity with minimal side-effects in animals. Estimation of human effective-dose.	Testing of drug on healthy volunteers for dose-ranging. In some case (*i.e.* oncology) testing on people in therapeutic failure.	*2a* Proof of concept study. Confirmation of the pharmacological efficacy on 50–200 healthy volunteers. Short-term exposition to the drug and one-dose testing. Highly-toxic drug directly go to phase 2b.	*2b* Looking for the optimum dose at which the drug shows maximal biological activity with minimal side-effects. Determination of the best route of administration. Determination of adverse effects and potential drug-drug interaction. Study on 100–300 people with the disease/ condition.	Pivotal study. Demonstration whether or not the drug offers a treatment benefit to a specific population. Looking for potential long-term or rare side-effects.	Postmarketing surveillance. Long-term study of the efficacy. Reporting of long-term or rare side-effects.
Duration	**Up to 6 years**		Several months	Several months to 2 years		1–4 years	
			From 5 to 10 years				
Global cost	**800,000,000 $ to 2,000,000,000$**						

shipment all-over the world, it could be possible for several States to share their stock of drugs; in this case each State could stock a drug against a different pathogen. With the recent Ebola crisis in Africa, the pharmaceutical industry also began to be aware of the fact that emerging diseases of biodefense interest could also occur naturally and be profitable. Another challenge is that the therapeutic window of the antibodies is short. The development of new antibodies (or the engineering of existing antibodies) that can cross the cell-membrane (transbodies) would be interesting; for example, transbodies directed against Ebola virus VP40 protein were developed [119]. Transbodies would be helpful after a massive terrorist attack, because the saturation of medical facilities is likely and in this case some victims may be treated only some days after their contamination. Considering the saturation of the medical facilities, the development of system for easy auto-administration of antibodies is strategic. For example, in the context of a project co-funded by the French ministry of defense, a drug delivery system for efficient alveolar delivery of ricin-neutralizing antibody was developed [90, 99]. This system would be helpful for an easy auto-administration both by the patient in a hospital context or by a soldier in a warfare context.

9.3 Conclusion

Biological agents have been used in warfare and terrorism many times in the past. A major threat for biodefense is that, with recent progress in biology and biotechnology, it is now possible to engineer biological agents to make more devastating bioweapons. Currently, bioweapons are more likely to be used by terrorists than by any nation state. Antibodies are known to be effective in the treatment of many diseases, including some of the diseases that might be caused by biowarfare agents. Many antibodies that neutralize biowarfare agents have already been isolated but only a few of these are either licensed or in clinical development. Efforts should be made to develop antibodies against new targets and finance the clinical development of those that have already been isolated. Some diseases are rare and developing antibodies to treat them is not considered a priority by the pharmaceutical industries. In this context, partnerships between commercial parties and States should be initiated to fund clinical development and antibody production to ensure strategic stockpiles of antibodies that could be vital for biodefense.

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