Chapter 28

FUTURE PROSPECTS OF VACCINES AND ANTIBODIES IN BIODEFENSE

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INTRODUCTION

Novel and next-generation vaccine approaches are being developed in parallel with new generations of antibody-based therapeutics. Each of these approaches has advantages specific to the disease, timeline, development status, and therapeutic or treatment window of its targeted pathogen. Usually, a specific or broadly neutralizing antibody response is required to provide either innate protection or vaccine-mediated protection, but this is not always the case. The increasing demand for antibody-drug conjugates, vaccine and small-molecule synergistic effects, and vaccine prophylactic use highlights the need to develop these two strategies individually and in combination to discover the optimal forms of protection.

Edward Jenner's 1794 discovery of the cross-protection afforded by a cowpox virus to variola virus led to the first vaccine, and ultimately to the successful eradication of variola on May 8, 1980, when the World Health Assembly certified the world free of naturally occurring smallpox. In the early 1880s, Louis Pasteur led the development of live attenuated vaccines, and in the late 1890s Emil von Behring and Kitasato Shibasaburo developed serum therapy against diphtheria and other microbes. Serum therapy was effective, but the administration of large amounts of animal proteins often led to undesirable side effects such as serum sickness. Within the same time period, vaccine developments provided efficient protective active immunity against rabies, tuberculosis, typhoid fever, and diphtheria and tetanus toxins.

The use of serum therapy declined after the discovery of antibiotics in the 1940s. It was later recognized that the critical protection afforded by crude serum was linked to polyclonal antibodies (pAbs), the "magic bullets" imagined by Paul Ehrlich. The development of biotechnology during the 20th century allowed Georges Köhler and César Milstein to isolate the first murine monoclonal antibody by immortalizing B cells in 1975. Hybridoma technology revolutionized antibody therapeutics and was later enriched by immortalization of human peripheral B cells, direct cloning of variable genes into phage expression libraries, and creation of transgenic mice that carry human antibody gene loci.[1]

The growing problem of increased bacterial resistance to antimicrobials, together with the need for alternative strategies to treat infectious diseases resistant to therapeutics, has stimulated a renewed interest in antibody therapy in the fields of infection and intoxication within biodefense. Although the risk of antimicrobial resistance is acute, an agent selected for deliberate dissemination may also be selected or engineered for antimicrobial resistance to existing treatments. Antibody therapy may be one way to mitigate that risk. Furthermore, utilization of antibodies in combination with existing therapeutics may provide synergistic benefits.

The pathway for monoclonal antibody (mAb) use in infectious disease follows proof-of-concept studies utilizing species matched fractionated serum, the use of human convalescent serum, and the early success of pAbs. Although initial results are promising, only a few mAbs are biodefense-related; namely raxibacumab, which obtained approval by the Food and Drug Administration (FDA) in 2012 for treatment of inhaled anthrax. However, several biodefense-specific mAb cocktails are showing success in early clinical phase trials.[2] The FDA has approved antibody therapy for emergency use when no other therapeutic options are available.[3] Throughout this chapter, monoclonal antibodies will be abbreviated as mAbs. Some of these therapeutics are also abbreviated as rAbs, or recombinant antibodies, in other texts, defined by their selection or engineering, and expression utilizing a range of DNA-based molecular biology techniques. Here, novel antibody designs (ie, fusion proteins) will be identified by their construct.

Vaccine development has also greatly benefited from advanced biotechnology. New vaccines may reveal previously unrecognized or underappreciated pathways to providing protection against biological weapons. This chapter will first examine the possible future of vaccines in a biodefense perspective, then consider how antibodies may provide novel and efficient ways to protect soldiers.

TRENDS IN BIODEFENSE VACCINE DEVELOPMENT

The FDA has fully licensed only two biodefense-related vaccines (Table 28-1). The development of vaccines is generally an expensive undertaking; a single vaccine usually takes 10 to 15 years to reach licensure, at a cost estimated in the hundreds of millions of dollars.[4] The pharmaceutical industry's evaluation of the cost is not simple, but a recent rotavirus vaccine is estimated to have cost between $205 million and $878 million.[5] For vaccine manufacturing companies, the biodefense market is limited, and as defense budgets globally tend to decrease, decisions to develop novel vaccines will be carefully examined. However, healthcare authorities are increasingly recognizing the benefit and cost-effectiveness of vaccines, so vaccine research in biodefense makes sense.

TABLE 28-1

MAIN VACCINE STRATEGIES APPLIED TO BIODEFENSE AGENTS UNDER INVESTIGATION OR AUTHORIZED BY THE US FOOD AND DRUG ADMINISTRATION

Strategy	Vaccine	Agent	Manufacturer	Status
Live attenuated vaccine	Vaccinia virus	Smallpox	Sanofi (Paris, France)	Authorized by FDA
	Francisella LVS strain	Tularemia	DynPort Vaccine Co LLC (Frederick, MD)	Under investigation
	Stern strain	Anthrax	Colorado Serum Co (Denver, CO)	Not authorized by FDA
	EV76	Plague	Not currently manufactured	Not authorized by FDA
Recombinant vaccine vectors	c-Ad3-EBO	Ebola virus	NIAID-GSK (Brentford, UK)	Under clinical investigation
	VSV-EBOV	Ebola virus	NewLink Genetics (Ames, IA); Merck (Summit, NJ)	Under clinical investigation
Subunit vaccine	AVA	Anthrax protective antigen	Emergent BioSolutions, (Rockville, MD)	Authorized by FDA
	LcrV-F1	Plague	Dynport Vaccine Co LLC	Under clinical investigation

AVA: anthrax vaccine adsorbed
FDA: Food and Drug Administration
LVS: live vaccine strain
NIAID-GSK: National Institute of Allergy and Infectious Diseases-GlaxoSmithKline
VSV-EBOV: vesicular stomatitis virus-Ebola virus vaccine

The future of vaccine development can be seen as responding to two different demands: On one hand, health authorities are pushing for safer, well-defined, purified vaccines, including vaccines with minimalist compositions that are able to protect against a single agent. Future biodefense vaccines developed in this context will need to follow these requirements. On the other hand, defense authorities want vaccines effective against dozens of different agents, protective against pulmonary contamination, triggering a rapid immune response, easy to administer, easy to produce on a large scale, and stable enough for long-term storage. In addition to these specifications, development and production costs should be kept as low as possible. These two global trends are clearly antagonistic, but emerging technologies may help in the development of safer vaccines with a larger range of efficacy. This section will examine how emerging technologies may improve old vaccines or give rise to new, multivalent vaccines.

Live Attenuated Vaccines

Jenner's serendipitous discovery of an attenuated vaccinia virus (VACV)[6] was later rationally extended and developed by Pasteur to produce vaccines against anthrax[7] and later rabies.[8] Live vaccines have since proven to be very efficient, as evidenced by the eradication of smallpox,[9] the prevention and control of poliomyelitis[10] and yellow fever,[11] and, more generally, the control of infectious diseases throughout the 20th century. The only attenuation technique available before the 20th century was the time-consuming and rudimentary passage of virus in transformed culture cell lines or in atypical hosts. Ideally, live attenuated vaccines are composed of live virus or bacterium that establishes a mild infection at the site of replication, which the immune system then controls by mounting an immune response. Live attenuated vaccines use a weakened agent, allowing for a controlled infection, which is why these vaccines optimally activate immune effectors and are more effective than inactivated vaccines.[12] Live attenuated vaccines must respect a delicate balance. They should be active enough to be immunogenic, but also sufficiently attenuated to be safe. The present regulatory environment imposes strong limitations on the development of new live attenuated vaccines to limit the risk of wild type virulence reversion, especially for virus-based vaccines. Discussed later are promising technologies that may aid in the development of live attenuated viruses as safer vaccine candidates.

Replication-defective viruses represent the first approach to improving safety and limiting the risk of uncontrolled infection in an immune-compromised host. As mentioned previously, this attenuation technique has historically occurred through serial passage; for example, a VACV was passed more than 570 times in chicken embryo fibroblasts, leading to the production of an attenuated vaccine called modified vaccinia virus Ankara (MVA) in the 1970s.[13] Through passage, MVA lost approximately 15% of its genome, was rendered replication-defective, and thus has proven to be safe in large clinical studies. Capitalizing on sequencing technology, researchers have synthetically developed an MVA homolog on a Lister strain vaccine virus background by deleting five regions similar to the regions lost by MVA.[14] This demonstrates one way that genetic-based technology can help in the development of rational vaccines. These viruses, however, are less immunogenic than their parental strains, illustrating the delicate balance needed between immunogenicity and safety. Because they are defective in replication, these vaccines are too efficiently eliminated, leading to a poor immune response.

Codon deoptimization is a novel strategy for developing live attenuated vaccines against RNA viruses, which represent a unique challenge due to their high genetic instability. Numerous studies have shown how the low-fidelity, RNA-dependent RNA polymerase can allow a virus population to rapidly drift or potentially revert any mutation.[12] Mammalians, as well as many other organisms, have codon bias, using synonymous codons more frequently than others. This bias is probably correlated to the efficiency of translation, and observed in virus structural genes. Codon deoptimization in the genome capsid of poliovirus induced a profoundly attenuated strain that triggered a strong immune response in animals.[15] As a general strategy for other vaccine development, codon deoptimization offers the major advantage that the genome is not mutated; therefore, it does not affect the antigenicity of the proteins. Codon deoptimization has been applied to different RNA viruses[16–18]; however, more studies are needed to evaluate whether this promising approach is safe and applicable for large-scale production.

Another strategy for the production of live attenuated vaccines is using microRNAs (miRNAs), which are genetically encoded short RNAs that have tissue-specific or developmental expressions and that play a large role in gene regulation.[19] Researchers have inserted miRNA sequences into the poliovirus genome to restrict its replication in the central nervous system (CNS).[20] However, it cannot be ruled out that the virus, by replicating outside of the CNS, could insert mutations in the miRNA sequence and revert its virulence.

Finally, zinc-finger (ZF) nuclease-controlled live attenuated virus is another experimental strategy that may be exploitable in the future as a vaccine.[12] This strategy has used ZF domains coupled to other functional domains to produce novel transcription factors that increased or decreased gene transcription with promoter specificity.[21–23] In other studies, ZF domains were fused to a restriction enzyme nuclease domain, resulting in the cleavage of specific sequences of double-stranded DNA.[24–26] This strategy could be useful in vaccination efforts to prevent or eliminate persistent viral infections.

The first live attenuated bacteria-based vaccine for human use was developed against tuberculosis through 230 serial passages of *Mycobacterium bovis* over a period of 14 years on artificial medium by Calmette and Guérin.[27] Live attenuated vaccines have historically been developed by the empirical technology of passage, and used in humans against anthrax (in the former USSR)[28] and plague (in the former USSR and in developing countries where plague is endemic),[29] but they are not authorized in Western countries. *Francisella* live attenuated vaccine live vaccine strain is licensed by the FDA, but could be improved. Advances in molecular biology and a better knowledge of host–pathogen interactions will help in the future development of rational strategies for novel live attenuated bacterial vaccines.

Recombinant Vaccine Vectors

Viruses can be engineered as carriers of heterologous antigens. This was elegantly demonstrated more than 30 years ago using a VACV expressing the hepatitis B surface antigen (HBsAg), which induced a heterologous protective immunity against hepatitis B in chimpanzees.[30,31] Although very promising, this strategy has yet to lead to a licensed vaccine for use in humans. Many viruses have been tested for their utility as a vaccine vector through their capacity to express heterologous proteins and induce an appropriate immune response. The myriad virus genera used to express recombinant protective antigen (PA) of anthrax can be seen as a paradigm of the extensive possibility of molecular biology in vaccinology. Anthrax PA has been successfully expressed by members from very diverse families of viruses, including poxvirus,[32] rabies virus,[33] hepatitis B virus,[34] adenovirus,[35] and influenza virus,[36] as well as in baculovirus and vaccina virus recombinants.[37]

Interest in the virus vector strategy was recently tested by the quick development of vector vaccines to help control the 2014 Ebola virus outbreak in West Africa. A replication-defective chimpanzee adenovirus

type 3 vector (cAd3-EBO) vaccine has shown interesting preliminary results in phase I trials,[38] while a vesicular stomatitis virus-based vector will soon enter the clinical phase.[39] Only a limited number of viruses have successfully undergone large-scale manufacturing practices and clinical trials and may one day reach human licensure. These include poxviruses, adenoviruses, Alphaviruses, and Flavivirus.[40–42]

Poxviruses represent good vector candidates because they grow to high titers, are very stable when lyophilized, and can accept very large transgenes due to their large genome sizes.[43] Moreover, the issue of preexisting immunity is limited because the adult population born after 1980 is naïve, and the protection afforded by the vector against variola provides dual-purpose use in a biodefense perspective. This last point has been exemplified by the development of a bivalent vaccine of a VACV vector expressing anthrax PA that provides immunity against anthrax and variola.[32] MVA has been used as vector for multiple viruses, such as human immunodeficiency virus (HIV), influenza, and dengue, but it has been largely supplanted by avipoxviruses, especially canarypox, which have a better safety profile because they do not replicate in mammalian cells.[44] Canarypoxvirus-vectored vaccines have been developed against rabies and measles and studied in HIV.[44] Although they proved to induce significant immune responses, the immune response is reduced in hosts with preexisting cross-protective immunity against the vector. This relatively new field would benefit immensely from a greater understanding of precisely which viral genes are crucial to triggering an efficient immune response.

Human adenoviruses (Ads) are also live vectors well suited for vaccine development, with a large genome of 38 kb of double-stranded DNA.[41] Recombinant Ads vectors have the E1 segment deleted, allowing the insertion of 5 kb of heterologous antigens, and are replication-defective in human cells. Most Ads vectors have been developed using Ad5, Ad26, and Ad35.[41,45,46] Ad5 vectors can be administered by nasal spray, allowing targeted vaccination of the mucosal surface. The main drawback of Ads vectors is the presence of preexisting immunity for Ad5 and the recombination hazard during the production process.

Another viable vector platform is virus-like replicon particles (VRPs). VRPs have been generated using the members of the Alphavirus or Vesculovirus genera. Alphaviruses contain a single-stranded, positive RNA of 12 kb and are members of the family *Togaviridae*. Replicons derived from Sindbis virus, Semliki Forest virus, and Venezuelan equine encephalitis virus (VEEV) were engineered by replacing structural genes with heterologous genes.[47] Vesicular stomatitis virus-based VRPs expressing Marburg virus glycoprotein-induced immune cross-protection against various strains of Marburg virus.[48]

Yellow fever virus (YFV) was developed under the ChimeriVax technology by Sanofi (Paris, France) as a chimeric virus for other Flaviviruses, such as dengue, West Nile virus, and Japanese encephalitis virus.[42] The platform consists of the live YFV 17D vaccine backbone virus deleted of its premembrane and envelope genes, which were replaced by heterologous genes from other Flaviviruses. This vector has been closely examined regarding its safety and the risk of recombination in the environment. Although safe and tested in large phase III clinical trials for dengue, this technology is limited to the *Flaviviridae* family.[49]

Bacteria can also be used as a vector for carrying vaccine antigens. *Listeria monocytogenes* and *Salmonella* species have been used as vaccine vectors in numerous clinical trials, mainly as cancer vaccine candidates.[50] *Salmonella* has shown some promise from a biodefense perspective as a vector for vaccines against pathogens such as anthrax[51] and plague[52]; however, for a vaccine strategy in a general population, less pathogenic strains are desired.

Among many models, lactobacilli are regarded as interesting bacteria because they can induce a mucosal immune response by expressing an antigen locally. It has been shown that PA from *Bacillus anthracis* expressed by a *Lactobacillus gasseri* induces significant protection against the infection in mice.[53] Because lactobacilli are probiotic, they can be administered easily and carry multiple genes, providing potential immunity against diverse agents.

Additionally, *Bacillus* spores can be used as an antigen carrier and vaccine platform.[54] This platform is promising because the spores are very stable and can be used as a mucosal vaccine. As an example, *Bacillus subtilis*-expressing PA has been shown to induce protective immunity against anthrax in a murine model.[55]

One of the main hurdles for vaccine vectors is the evaluation of efficacy in a human population that may have acquired various levels of preexisting immunity. Limited data has been published so far on this topic, but a recent review of the literature suggests that for *Salmonella* vectors preexisting immunity can enhance subsequent induction of immunity, while for adenovirus preexisting immunity is a hindrance.[56]

Subunit Vaccines

Since the inception of vaccinology, the hunt for ways to address attenuated vaccine safety concerns has resulted in the search for immunogenic subunits. This was first exemplified in the 1920s by the

discovery that formaldehyde-inactivated exotoxins are immunogenic.[57] However, beyond exotoxins, large-scale production of subunit antigens was not feasible at the time. During the "golden age" of vaccinology, in the 1960s and 1970s, most vaccines were inactivated viruses or bacteria. Renewed interest in the development of subunit vaccines occurred in the 1980s with the emergence of a hepatitis B virus subunit vaccine composed of the HBsAg protein, initially purified from patient sera and later produced by genetic engineering.[57] Subunit vaccines are attractive for use in biodefense because they offer stimulation of targeted immune responses with minimal side effects. Indeed, the anthrax PA-based vaccine BioThrax (Emergent BioSolutions, Rockville, MD) and next-generation plague fusion protein LcrV-F1 vaccine currently under clinical investigation are both antigen-targeted subunit vaccines produced through genetic engineering.

First demonstrated 35 years ago, synthetic virus-like particles (VLPs) are molecular structures based on viral protein subunits that form artificial capsid units.[58] VLPs mimic native virions by displaying repetitive subunits of the viral surface proteins that present the immune system with conformational viral epitopes that can elicit strong T cell and B cell immune responses; however, they can be distinguished from virus by the absence of nucleic material. The absence of replication renders them safe antigens for use as a vaccine. In 1986, at the dawn of recombinant technologies, HBsAg was produced with yeast to form the first synthetic type of VLP to come on the market (Recombivax B [Merck, Summit, NJ] and Engerix B [GlaxoSmithKline, Brentford, United Kingdom]). Some 20 years later, two human papilloma virus (HPV) vaccines (HPV 16 VLP vaccine and HPV 18 VLP vaccine)—based on the L1 protein produced in yeast or insect cells—were licensed (Cervarix [GlaxoSmithKline] and Gardasil [Merck]).[59] More recently, a VLP-based hepatitis E virus vaccine was licensed for use in the Chinese market (Hecolin [Xiamen Innovax Biotech, Xiamen, China]).[60]

Numerous VLP-based vaccines are currently in preclinical development and clinical trials, which shows the potential of this strategy.[61] Of particular interest for biodefense, VLPs have shown promising initial results in protection against the Ebola and Marburg viruses.[62] VLPs consisting of up to three antigens—(1) the glycoprotein, (2) the matrix protein (VP40), and (3) the nucleoprotein—as well as "hybrid VLPs"—containing both Ebola and Marburg glycoprotein and nucleoprotein—can confer protection against homologous challenge with either virus in rodents and nonhuman primates.[62] VLPs can be genetically engineered, incorporating peptides, proteins, or pathogen-associated molecular pattern ligands to create elaborate scaffolds.[63]

The plasticity and potential of VLPs are quite remarkable and limited only by the human imagination. Their plasticity is exemplified by insect Flock House virus VLP nanoparticles that display anthrax antigen. When the VLPs are coated with the PA-binding domain of anthrax toxin receptor 2, they serve as a decoy receptor for anthrax toxins in vitro and in vivo. Alternatively, when coated with PA protein, the VLPs can be used as an efficient anthrax vaccine.[64] VLPs can be used as a molecular platform to help the immune system recognize heterologous antigen. These characteristics place synthetic VLPs at the boundary between subunit antigen and adjuvant (see the discussion under Adjuvants).

Initially launched in the 1980s with the first pneumococcal vaccine, recent technological advances in glycan synthesis, glycan structure analysis, and glycochemistry are paving the way for a new era in carbohydrate vaccine design.[65] In this approach, the surface of bacteria is covered by a dense array of polysaccharide on the lipopolysaccharide and the capsule, forming a unique feature with strong antigenic properties. It is important to note that glycan synthesis is complex and its support of antigenic specificity is not genetically encoded. The conjugation of glycans to a carrier protein or to an adjuvant induces long-lasting protection against encapsulated bacteria, which could potentially be extended to viruses. This novel and booming field may help develop new vaccines against gram-negative or capsulated bacteria such as *Yersinia pestis, Francisella tularensis,* and *Burkholderia pseudomallei*.

Advances in genomics, including high-throughput DNA sequencing, have provided access to complete antigenic repertoires of pathogens. Capitalizing on this information, in a process coined "reverse vaccinology," previously unknown vaccine antigens have been identified.[66,67] The first success story of this strategy was the long-sought vaccine against meningococcus B.[68] In contrast to *Neisseria meningitidis* serogroups A, C, Y and W135, carbohydrate vaccines could not be used for serogroup B due to the similarities between the capsular polysaccharide and a human neural antigen. The first step of the lengthy process of developing this vaccine was a computer analysis of the targeted genome sequence to identify all genes coding for predicted antigens. The 600 predicted antigens were screened for their expression by the pathogen and their immunogenicity (referred as the antigenome).[69] Twenty-nine selected antigens were further tested for their immunogenicity in animal

models. Lastly, the selected PAs were evaluated for their presence in worldwide representative pathogen collections. Five stable PAs were industrially produced for clinical trials. At each step of the process, the number of antigens dramatically decreased, but the power of the strategy comes from the complete genome analysis.[68] Although this strategy is costly, it may be applied to diverse biothreat agents for which other methods of vaccine development have so far been ineffective.

"Systems vaccinology" is a generic term recently coined to describe the use of systems approaches to identify signatures that can be used to predict vaccine immunity in humans.[70] The two major goals of systems vaccinology are to characterize the host response by identifying genes and pathways whose expressions are altered in those receiving vaccines, and then to identify predictors of vaccine efficacy. Systems vaccinology is still in its infancy, but it may help in the future design of efficient vaccines, especially for pathogens that constantly change their surface-expressed molecules, such as plasmodia and other eukaryotic parasites.[71]

Structural vaccinology, the use of three-dimensional structural information to design novel and improved vaccine antigens, is another trend in rational vaccine design. Advances in X-ray crystallography and nuclear magnetic resonance spectroscopy have enabled researchers to pinpoint new structures and antigenic epitopes at atomic resolution.[72] Recent advances may have solved one of the hottest issues in vaccinology: respiratory syncytial virus (RSV). RSV has been a challenge for vaccine development after a disastrous clinical trial in the 1960s showed that a killed vaccine enhanced the disease, increasing hospitalization rates of children and causing two deaths.[73] RSV fusion (F) glycoprotein, which helps the virus merge its membranes with the host cell, exists in two conformational states, the prefusion metastable state and the postfusion stable state.[74] By engineering soluble variants of RSV F with a stably exposed antigenic site, researchers have identified one easy-to-produce variant that induces a strong neutralizing immune response in murine and nonhuman primate models.[75] Complete understanding of antigenic structure may aid the identification of key sites to target in order to disable a protein's function, stabilize select conformations of a protein, or determine antibody–antigen complexes, all of which would allow the development of more stable, homogeneous, efficiently produced vaccine antigens. This strategy might be applied with great success to viruses for which neutralization could be obtained through surface glycoproteins, such as hemorrhagic fever viruses.

Nucleic Acid Vaccines

Proof-of-concept studies for nucleic acid vaccines occurred more than 2 decades ago, when it was shown that intramuscular injection of mRNA or DNA resulted in local production of a reporter gene[76] and the induction of an immune response.[77,78] For DNA vaccination with this approach, the gene of an antigen is encoded and expressed from a plasmid-based system. In theory this method provides a subunit vaccination; however, by expression of the antigen within the host, rather than exogenous supplementation of the protein, the antigen is expressed in its natural form. As a result, the antigen can be processed by the immune system to activate both humoral and cellular immune responses.[79] DNA vaccines are particularly attractive in the biodefense field because they offer many advantages: (*a*) they can be engineered without the need to culture a pathogen; (*b*) manufacturing processes to produce plasmid DNA are well-established; (*c*) plasmid DNA manufacture is extremely rapid and can be designed for any engineered or emerging pathogen; and (*d*) the vaccines are safe and pose no risk of integration into the genome.

Despite these obvious advantages and many clinical trials, no DNA vaccines have been authorized for human use by the FDA.[80] Many hurdles need to be overcome if there is to be a future for this vaccine class. One such challenge is the manufacturing of plasmid DNA on a large scale with good manufacturing processes.[81] In the biodefense field, DNA-based vaccines have been tested against anthrax with somewhat disappointing results, but they have shown efficiency against Filoviruses, poxviruses, and encephalitic Alphaviruses.[82] The greatest challenge, though, is the suboptimal immunogenicity elicited by the vaccines in humans, which has been found to be significantly lower than that observed in rodents. Several strategies have been developed to improve the vaccines' immunogenicity.[79] As with live vaccines, codon optimization has been tested and helps with transgene expression in human cells. Another strategy has been to optimize the design of the transgene, including adding untranslated regions which may be critical regulators of vaccine gene expression located 5′ and 3′ to the encoded gene. One additional strategy has been to include unmethylated cytosine-phosphate-guanine (CpG) motifs in the sequence. CpG motifs are absent from eukaryotic DNA and are recognized as pathogen-associated molecular patterns by toll-like receptor (TLR) 9. The presence of CpG motifs may increase immunogenicity by stimulating a robust innate immune response.

In addition to making alterations in the plasmid DNA itself, changes in the delivery of DNA vaccines may enhance the quality and magnitude of the immune

response elicited. Many technologies have been tested, and the debate over their efficiencies is as long-standing as DNA vaccine technology itself.[83] So far, needle injection (subcutaneous or intramuscular), particle bombardment ("gene gun"), high-pressure liquid delivery, and electroporation have been evaluated, and each has specific advantages and disadvantages.[83,84]

RNA vaccines have been developed more recently, mainly because RNA molecules were known to be less stable. Despite this instability, RNA vaccines retain three major advantages over DNA vaccines.[85,86] First, RNA must be delivered only into the cytoplasm of target cells (in contrast to DNA, which must be transported to the nucleus). Second, genomic integration of the RNA is not possible, circumventing this safety concern (as well as the risk of inducing anti-DNA antibodies). Thus, RNA vaccines are not classified by the FDA as "gene therapy." Third, RNA interactions with the host are very versatile because the intracellular host innate immune response is focused on the detection and elimination of exogenous (mainly viral) RNAs. Due to the transient nature of RNA and the requirement of translation by host machinery, the load of antigen produced is more controlled, thereby minimizing the risk of tolerance induction by long-term antigen exposure.

Proof-of-concept studies demonstrating the efficiency of RNA vaccines has been established for numerous antigens.[85–87] However, RNA vaccines are still very experimental and require improvements to overcome the hurdles of clinical vaccine development. RNA stability and delivery methods need to be improved. Ribonucleases are present on the skin and in tissues, and mRNAs are negatively charged, which impairs their entry in the cytosol through the cell membrane. New methods for improving the RNA transfection efficiency may help. A recent study has shown that encapsulating a self-amplifying RNA into a lipid nanoparticle protected it from ribonuclease digestion and elicited a broad, potent, and protective immune response in rodents.[88]

High production costs and low RNA yield from in vitro production of mRNAs presents the second major challenge to the development of RNA vaccines.[89,90] So far, the longest chemically synthetized RNA with biological activity is 117 nucleotides.[90] Next-generation nucleic acid vaccines will also require an improved delivery technology, assessed in conjunction with the development of the vaccine.

Mucosal Vaccines

During an attack, biothreat agents would likely be presented in one of two primary routes of administration: aerosols or introduction into food or water supplies. Thus, an infectious agent would enter the host through the respiratory or intestinal mucosae. For this reason, vaccines targeting the mucosal surfaces seem logical for biodefense. However, a distinction between agents that elicits mucosal infections and those that simply exploit mucosal tissues as a mean to gain access to the body should be made.[91] When developing a vaccine to protect the mucosal surfaces, two strategies exist: (1) to increase the mucosal response of existing systemic vaccines and (2) to design a vaccine targeted for mucosal delivery and immune response,: the latter will be examined in this section. Mucosal vaccine development should be focused on agents that provoke a mucosal-associated infection, but not on agents that are controlled by systemic immunity. Mucosal vaccines offer many advantages from a production and regulatory point of view[92]: (*a*) oral vaccines do not need purification steps because the gut microbiota is already complex; (*b*) mucosal vaccines do not require injection, so they are subject to a better compliance and are suited for mass vaccination as they do not require medical personnel for administration; and (*c*) mucosal vaccines do not carry risk of spreading blood-borne infection because no needles are involved.

Mucosal immunology is an expanding field that has led to a better understanding of the mucosal immune system and response. Nevertheless, few mucosal vaccines have made it to licensure. Thus far, the only live attenuated virus vaccine administered intranasally are FluMist (MedImmune, Cambridge, United Kingdom), against influenza virus, and Vaxchora (PaxVax, Redwood City, CA), a suspension of *Vibrio cholerae* strain CVD 103-HgR) against cholera.[93] Two other live attenuated vaccines administered orally against rotavirus[94] and *S typhi* have also been efficiently launched.[95] Notably, two inactivated oral vaccines against cholera have been marketed, providing an interesting proof-of-principle for future inactivated vaccines.[96] In the biodefense field, *Y pestis* has been a model for the development of live mucosal vaccines providing protection against bubonic and pulmonary plague.[91]

A major challenge for mucosal vaccine development is the production of candidates that strike a balance between immunogenicity and attenuation. Historically, most mucosal vaccines have been produced through passage of virus in host cell culture, as was done for the oral polio vaccine. It is clear that mucosal vaccines will benefit immensely from a more rational approach using genetic manipulation to increase their safety and stability, as exemplified by the typhoid Ty21A vaccine.[97]

Another area of mucosal vaccine development that needs attention is characterizing the optimal route of immunization. The biodefense community frequently

focuses on intranasal, oral, and sublingual routes of immunization. The mucosal immune system is compartmentalized into nasopharynx-associated lymphoid tissues, bronchus-associated lymphoid tissues, and gut-associated lymphoid tissues.[98,99] Although some functional connections between the respiratory and gut immune systems have recently been described,[100] the presumably limited connectivity between the respiratory tract and the gut places a constraint on the definition of the optimal route of immunization. As a result, different routes of immunization tend to result in compartmentalized responses, with (*a*) intranasal immunization inducing a strong immune response (measured by the secretory immunoglobulin A [IgA] production) in the upper and lower respiratory tract and the gastric and genital tracts; (*b*) sublingual immunization inducing a response in the upper and lower respiratory tract and the gastrointestinal tract; and (*c*) oral immunization triggering an immune response in the salivary glands, gastrointestinal tract, and mammary glands.[98,99]

Development of a strong immune response to oral vaccination is especially challenging because of oral tolerance and the host's need to maintain homeostasis to protect against immune responses to digested antigens. Even after preclinical success with oral vaccination, many clinical studies have failed to induce an effective immune response.[97,99] Therefore, oral vaccine formulation studies are needed to gain a better understanding of the effects of stabilizing the antigens in a harsh environment and targeting them to the gut-associated lymphoid tissues. Intranasal administration remains attractive because of the large mucosal surface area the nose provides and the access to the nasopharynx-associated lymphoid tissues, which can activate immune responses in the respiratory and gastrointestinal tracts.[99] The seemingly global mucosal stimulatory potential after intranasal immunization may represent an important path for novel vaccines. Moreover, intranasal administration lowers antigen and adjuvant doses compared with oral vaccinations, making them more cost effective. Aerosol spray, droplets, and powders have been optimized and represent an attractive field for the development of new devices.[101,102]

However, the primary role of the nasal mucosa is to protect the respiratory airways, not to convey antigens to the immune system.[101] A challenge for intranasal immunization is that the nasal mucosa is intricately connected to the olfactory nerve and the CNS. As a result, intranasal immunizations using *Escherichia coli* heat labile toxin as an adjuvant in humans has been correlated with Bell's palsy development.[103] In contrast, sublingual immunization has gained interest because it stimulates a broad activation of the immune system while avoiding perturbation of the CNS.[104]

Adjusting mucosal vaccine formulations may be another way to improve their efficacy. Little is known about how to formulate a better mucosal vaccine, although there are currently two main approaches: using either (1) soluble or (2) particulate vaccines.[92] Defining the most effective vaccine formulation is a universal challenge in vaccinology (see the discussion in Adjuvants).

Finally, the development of new mucosal adjuvants could significantly improve the effectiveness of mucosal vaccines. Stimulating the appropriate type of immune response can dramatically affect the immediate and long-term immune response to a vaccine, ultimately determining protection from disease. The use of heat labile enterotoxin and cholera toxin has been abandoned due to neurologic effects and overt diarrhea, respectively.[105] In the future, mucosal adjuvants may be improved by combining particles with TLR ligands.[106] Alternative adjuvants are discussed in the following section.

Adjuvants

The word "adjuvant" is derived from the Latin verb "adjuvare" meaning "to help." Adjuvant properties were discovered by Gaston Ramon in 1920.[107] Although adjuvants have proven to be crucial for most vaccines, the field has been relatively neglected until recently. The most widely used adjuvant to date, alum, has been empirically instilled in billions of vaccine doses since the 1920s, even though its mode of action was not discovered until 2008.[108] The only other category of adjuvant authorized for human use is the squalene derivatives: MF59 by Novartis (Basel, Switzerland),[109] AS03 by GlaxoSmithKline,[110] and AF03 by Sanofi-Pasteur (Lyon, France).[111] These adjuvants have been used in the pandemic influenza vaccine since 2009.[112] Over the past decade, many preclinical studies have expanded the list of potential adjuvants.[113,114] Historically, adjuvants have been recognized as a "perfect mix" of old ingredients, including water in oil (w/o) and oil in water (o/w).[115] Their adjuvant properties are thought to be based on nonspecific inflammatory stimulation that brings all the cellular players to the site of inoculation. Instead of the typical adjuvant formulations of the past, some large pharmaceutical companies such as GlaxoSmithKline have developed new mixes adapted for specific vaccines that require strong and long-lasting immune responses.[116] The rediscovery of the central role the innate immune response plays in the

development of overall immunity has encouraged the design of TLR and nucleotide-binding oligomerization domain receptor (or NOD-like receptor [NLR]) ligands as adjuvant components. For example, AS04, developed by GlaxoSmithKline, is composed of the TLR4 agonist 3-*O*-desacyl-4′-monophosphoryl lipid A (MPL), a component of lipopolysaccharide.[116] Interestingly, this bacterial component has proven to be a good adjuvant for virus vaccines such as the human papilloma virus vaccine Cervarix, demonstrating that viral and bacterial motifs can cooperate to activate the immune system. More intricate mixes have also been tested by GlaxoSmithKline, including AS02, which contains MPL and QS-21, a saponin-based adjuvant derived from the bark of *Quillaja saponaria*.[116]

In the same context, there is potential that the emerging nanotechnology field may help improve adjuvants in the future.[63] Nanoparticles (1–1,000 nm size particles) retain adjuvant activity by improving antigen delivery and triggering innate immune responses. Numerous polymer motifs, such as poly(lactide-co-glycolide) nanoparticles, co-polymers hydrogels, and cationic liposomes, have been tested as carriers of antigens. Some biodegradable nanoparticles are safe and already available for use in vaccine formulation.[117,118] These particles can be decorated by molecular motifs either to target specific cells, such as dendritic cells or macrophages, expressing specific pattern-recognition receptors. Nanotechnology may supply an opportunity to improve vaccine adjuvants used in biodefense.

THE FUTURE OF ANTIBODIES IN BIODEFENSE

Just as with vaccine discovery, the development of antibody-based therapeutics can be an expensive and time-consuming effort, with new products taking years to reach licensure; however, most of the more recent products are of human or human-like origin, often requiring less time than older products to reach this milestone. Antibody-based therapeutics represent the fastest growing class of biological therapeutics: 43 therapeutic mAbs had been approved as of December 2013, and 36 of these are still active in the European Union or US market.[119] However, few of these are specific to biodefense agents. Biodefense-specific infectious agents and toxins are generally not as well studied, limiting the targets and antigenic material required for antibody generation. The production of these therapeutics has predominantly been hindered by the historically high cost of antibody therapeutic development, primarily in the production of a final protein in the concentration and format necessary to elicit protection. Also, in contrast to the "one-bug, one-drug" approach often used for vaccines designed to combat multiple agents (utilization of a single drug for each agent), antibody development costs may be increased by the need for oligoclonals, or cocktails, of multiple antibodies that focus on a single agent, often acting via different mechanisms or protecting against escape mutants. Despite these constraints, antibody therapeutics are increasingly necessary to fill the gap when vaccine development has yet to produce an efficacious product, as in the 2014 West Africa Ebola virus outbreak.[120]

Antigen-specific protection afforded by antibodies can have advantages compared to the protection elicited by vaccines, especially for biodefense. Vaccines must elicit an effective, long-lasting immune response whose maintenance may require multiple booster injections. Antibodies, however, may be administered in quantities that achieve a titer of protection that exceeds that elicited by vaccines due to the decreased immunogenicity of antibodies when used in a human or humanized format. These mAbs may then provide a higher level of protection, a level necessary in biodefense, because bioweapon exposure is often intended to use elevated levels of the agent or toxin compared with natural exposures. Emerging diseases and toxins do not always represent a threat exclusively, and several agents have been evaluated for their therapeutic potential. For example, botulinum neurotoxins (BoNTs) have a therapeutic application, making vaccination clinically disputable. Antibodies can serve multiple roles: as a therapeutic alternative when no vaccine is available or as the sole treatment when a vaccine would not be efficacious. This section will discuss new and emerging technologies that have improved antibody discovery; events specific to antitoxin, antibacterial, and antiviral antibody development; and the future formats and production challenges of these molecules.

Antibody Generations and the Development of New Therapeutic Formats

The most representative and recognizable antibody format, immunoglobulin G (IgG), is often the simple fractionation or isolation of antibodies from human or animal sera to produce protective antibodies (Figure 28-1). Additional antibody formats will be discussed later in this chapter.

Sera, the first antibody product used, has recently seen a resurgence of use. Generally, sera is fractionated to produce pAbs that are used as a first line of defense

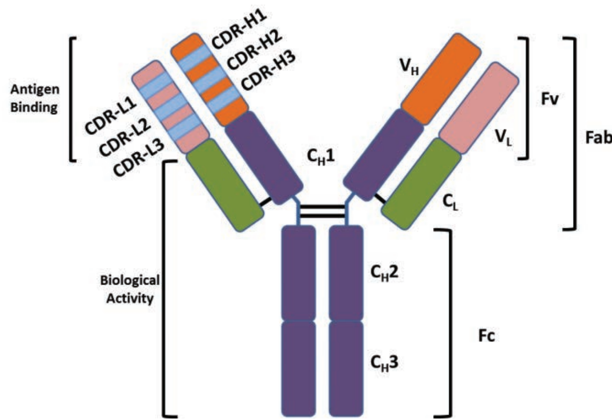

Figure 28-1. Representation of the immunoglobulin G antibody isotype. Specific regions are identified by color. The variable light (V_L) and variable heavy (V_H) regions, in *pink* and *orange*, respectively, together make up the Fv region. Each Fv and C_H1 domain (upper *green* and *purple* for each V domain) makes up a Fab fragment. The combination of the C_H2 and C_H3 (*purple*) domains makes up the Fc fragment.
Fab: fragment antigen-binding; Fc: fragment crystallizable; Fv: variable fragment

against emerging pathogens; pAbs have been essential to providing critical treatments for infectious diseases. The use of these pAbs, as well as their role as future therapeutic countermeasures, will be discussed later within each subsequent section.

Historically, mAbs have been associated with several advancing generations of molecules (Figure 28-2). The first generations of mAbs were strictly murine in origin, as described in the early 1980s by Köhler and Milstein, who used hybridomas from stable fusions of immortalized myeloma cells with B cells from immunized mice.[1] Several of these mAbs made it to clinical trials for the treatment of cancers and transplant rejection, but were ultimately withdrawn due to a variety of concerns, including the high immunogenicity of these foreign proteins in humans, first identified by responses from human anti-mouse antibodies.[121]

Due to the immunogenicity of these antibodies, murine mAbs had little utility and often caused adverse effects. Following the publication of Köhler and Milstein's hybridoma article, human/mouse chimeric antibodies were developed as second generation therapeutic molecules with a human Fc region to decrease immunogenicity.[1] Several biodefense mAbs have been produced in this format, primarily due to the ability to develop antibodies using mouse models for exotic infectious diseases that could be rapidly chimerized to a human Fc region for a single clinical application. Even with the addition of a human portion to the antibody, the administration of these chimeric antibodies continued to produce an immune response to the remaining murine domains.

Third- and fourth-generation mAbs were developed by using selection and engineering advances in recombinant DNA-based molecular biology techniques to reduce immunogenicity. This was accomplished through humanizing the framework regions and transferring only the antigen-binding loops made up by the complementary-dependent regions. Further humanization can be achieved by making point mutations within the complementary dependent regions themselves. Humanization of each mAb generation requires special care because multiple residues of the framework regions are often required for stability of the antibody; therefore, it is essential to ensure that these modifications do not negatively impact the binding or activity of the mAbs when changing residues within the complementary dependent regions. Although the overall immune systems of mice and

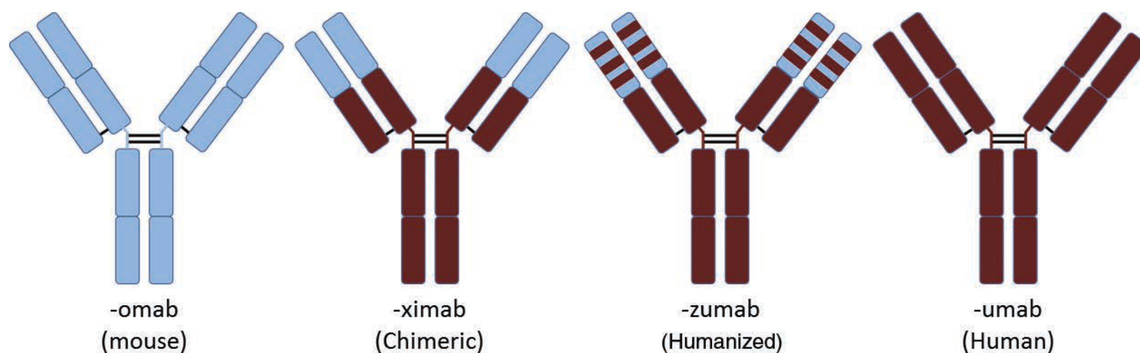

Figure 28-2. Representations of the progression of antibodies through the progressive generations of humanization from mouse (-omab), chimeric (-ximab), humanized (-zumab), and human (-umab) antibodies.

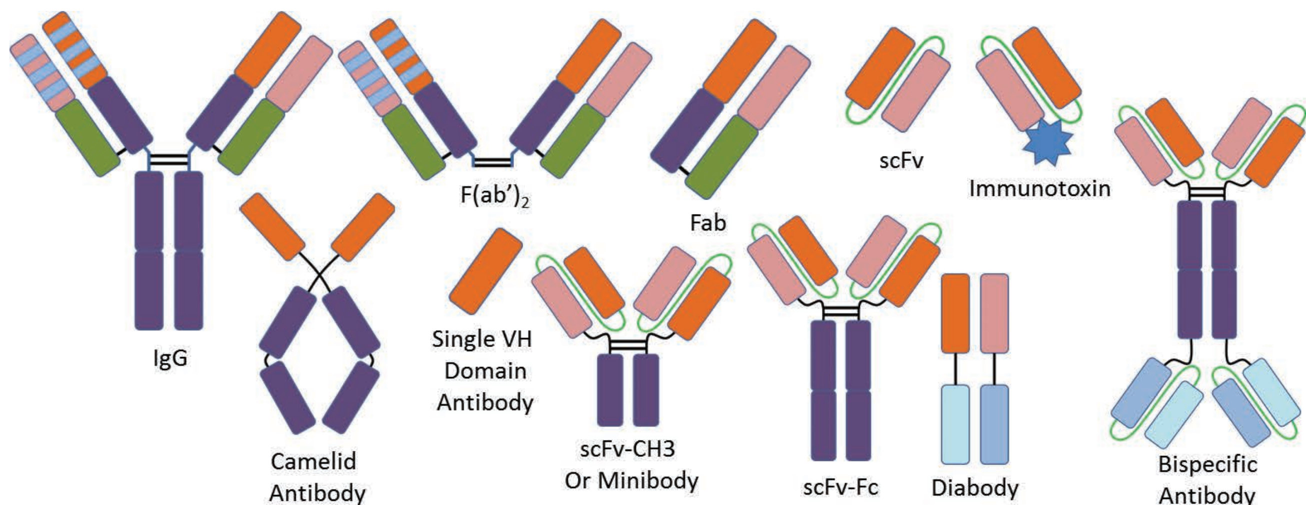

Figure 28-3. Representative antibody fragments demonstrating therapeutic potential through particular function, access, or host interaction. Immunoglobulin G (IgG) shown on *left* for reference.
Fab: fragment antigen-binding; Fc: fragment crystallizable; scFv: single-chain variable fragment; VH: variable heavy
Illustration: Adapted with permission from Frenzel A, Hust M, Schirrmann T. Expression of recombinant antibodies. *Front Immunol*. 2013;4:126. This work is licensed under a Creative Commons Attribution 3.0 Generic License (https://creativecommons.org/licenses/by/3.0/legalcode).

humans are similar, researchers should understand that the response in mice might not always correlate to the human response.[122] The most recent techniques used to produce human or humanized mAbs include DNA-based selection libraries (eg, phage display[123]), isolation from peripheral blood B cells,[124] and use of transgenic humanized animals.

The future of antibody development may not be in the isolation of the antibody to obtain a fully human product, but rather in the production of new formats that are able to enhance a particular antibody function, or in the administration of the antibody to the target or host. New mAb therapies being explored use not only antibody–vaccine combinations, but also antibody–drug conjugates[125] and many new antibody-based formats, such as bispecific and multispecific antibodies,[126] intrabodies,[127] and transbodies[128] (Figure 28-3).

The IgG1 isotype, depicted in Figure 28-1, is the most abundant isotype of immunoglobulin produced against protein antigens. Other isotypes include IgA, IgD, IgE, IgM, and multiple subclasses of IgG. As therapeutics, IgGs have seen the greatest use because they are the most predominant and abundant antibody in the serum and have historically been easier to isolate and purify. IgMs are large-molecular-weight pentamers and are the first low-affinity antibodies expressed in response to an infection. These antibodies are secreted from the surface of B cells before their maturation into a plasmocyte prior to the IgG isotype switch. The lack of recombinant and isolated IgMs available as therapeutics may predominantly be due to their overall molecular weight and the subsequent difficulty of purifying them.

IgAs consist of monomers in human serum and dimers after secretion at the mucosal surface through polymeric Ig receptors, are most prominent within the mucosal and gastrointestinal tract and serve as the first line of defense against natural infections.[129] The lack of alternative antibody isotypes beyond IgGs available for use against therapeutic targets can partly be attributed to the lack of appropriate in vivo models and partly to the difficulty in purifying these products in their dimer form. New transgenic species and advanced antibody production methodologies will most certainly provide the necessary tools to test alternative classes of antibody therapeutics.

IgG antibody isotypes, and to a lesser extent, fragment antigen-binding (Fab) fragments, have been the primary therapeutic format of antibodies that have been FDA approved for use. These antibodies have an extremely large size (around 150 and 55 kDa, respectively) and a half-life averaging 21 days for a human antibody, but they may not have optimal presentation to the epitope or tissue. Functional CH2 domains (called nanoantibodies),[130] single-domain heavy chain (abbreviated VHH) camelid-derived nanobodies,[131] and fusion antibody fragments are progressing through late stage development and into clinical trials. As in the case of single domain nanoantibodies and nanobodies, these smaller, independently folded domains with antigen

binding capabilities may serve as highly specific and extremely stable therapeutics. Although they possess remarkably stable physiochemical properties, these antibodies are rapidly cleared from the bloodstream in a matter of hours, hindering their development as a potential therapeutic in their base format. Methods to increase the half-life of these antibodies include binding and linkage to Fc domains, albumin, and polyethylene glycol. For both full-length antibodies and new single-domain formats, a range of methods is being developed to increase the antibodies' serum half-lives.

Antitoxin Monoclonal Antibodies

Treatment for intoxication using mAbs has seen a resurgence of utility in the past several years. Early work with serum therapy and passive transfer of antibodies provided successful proof-of-concept studies against diphtheria and tetanus via toxin neutralization.[132] Subsequent development of antitoxin antibodies, using high antibody affinity as a primary discriminator for selection, has shown advances against bacterial and viral targets.[133–135] Selection for these affinities has spurred vast improvements in anti-ricin and anti-BoNT, often into the sub-nM and pM levels. However, the narrow specificity of the antibodies to the toxin targets often limits their efficacy when used against toxins with multiple serotypes, as with BoNT, which has seven known serotypes (A through G).[136] Selection of these antibodies has been further driven by improvements in humanization. This section will primarily focus on toxins that have the capability for weaponization as biowarfare toxins in the absence of the producing pathogen.

Ricinus communis, a plant extensively cultivated around the world produces the Ricin toxin. Ricin is a type 2 ribosome-inactivating protein consisting of two parts. The B-subunit (RTB) binds to sugars on the cell surface for cytosolic entry of an A-subunit (RTA), which inhibits protein synthesis. Both of these subunits of the toxin have epitopes that have been targeted for therapeutic and vaccine development. Although two vaccines are undergoing early phase clinical trials (NCT01317667 and NCT00812071), there are currently no licensed measures for treatment, leaving options for supportive care only. (See Table 28-2[137–145] for more details.) A proof-of-concept study using direct inhalation of ricin toxin followed by treatment with pAbs 20 minutes postchallenge demonstrated protection.[146] Monoclonal antibody development has progressed through multiple antibody formats against both RTA and RTB.[147,148] Chimeric mouse/human antibodies are currently the only format that has been successfully used to demonstrate protection postintoxication.[141,149] Future use of antibodies against ricin intoxication may require a mixture of antibodies to both RTA and RTB to provide protection against the multiple mechanisms of intoxication. Humanizing these early protective antibodies will be essential in reducing the therapeutic agent's immunogenicity and expanding its pharmacokinetic capabilities.

Clostridium botulinum is a gram-positive, spore-forming, anaerobic bacteria that secretes neurotoxins causing botulism.[150] *C botunlinum* produce multiple serotypes of the neurotoxin BoNT, from A to G (denoted BoNT/A to BoNT/G), of which, types A, B, E, and F have been shown to cause human botulism.[136] In 2011, the Centers for Disease Control and Prevention discontinued the investigational new drug (IND) use of the pentavalent (ABCDE) botulinum vaccine, leaving a therapeutic gap in the protection of at-risk workers.[151] Although most of the treatments for botulism are based on supportive measures (eg, intensive respiratory care), passive administration of anti-BoNT immune sera has been used for treatment. A civilian working group on biodefense recommended the utilization of antisera immediately following diagnosis,[152] and treatment with equine antisera within 24 hours has demonstrated success.[153] Two therapeutic products have been derived from equine pAbs for biodefense use.[137] Well-suited to protect against the various serotypes of BoNT, these formulations were prepared, although not used, during Operation Desert Storm and Operation Desert Shield in the 1990s. The new heptavalent botulinum antitoxin (HBAT, Cangene Corp, Winnipeg, Canada) is composed primarily of Fab and $F(ab')_2$ fragments and is available from the Centers for Disease Control and Prevention under an IND protocol for naturally-acquired botulism.[137] New formulations of oligoclonal mixtures are being developed to recapitulate broad serotypic capacity, providing a more efficacious product and extended half-lives in humanized formats. Two parallel efforts, currently under development by the United States and European Union, are to develop a panel of antibodies against BoNT/A/B and /E (XOMA 3AB and AntiBotABE, respectively).[139,147,154]

Even given the rapid clearance and success of polyclonal and novel monoclonal antibody mixtures, the antibodies are effective only against the circulating toxin, and often against just a single serotype or subtype each. Once the toxin is attached to the receptor, it is internalized by receptor-mediated endocytosis, ultimately blocking neuromuscular communication. However, the administration of antitoxin may still be necessary because, even after the onset of symptoms, BoNT may remain in the bloodstream throughout infection, and clearance is essential for subsequent recovery. The oligoclonal approach to developing a cocktail against multiple serotypes is necessary for any future

TABLE 28-2

EMERGING ANTIBODY-BASED BIODEFENSE THERAPEUTICS

International Nonproprietary Name, Product Name, or Code	Targeted Agent	Targeted Antigen	Category*	Antibody Type (Isolation)	Approval, Clinical Phase, or Stage	US Patent or Reference No.
HBAT (Cangene Corp, Winnipeg, Canada)	*Clostridium botulinum* toxin (botulism)	BoNT (A–G)	A	Polyclonal Fab and $F(ab')_2$ fragments	IND treatment	(137)
XOMA 3AB (Xoma Corp, Berkeley, CA)	*C botulinum* toxin (botulism)	BoNT (A)	A	Recombinant IgG1 mAbs (human and murine origin)	Phase 1	NCT01357213
AntiBotABE (EU Consortium, 7th Framework Programme, Brussels, Belgium)	*C botulinum* toxin (botulism)	BoNT (A)	A	Recombinant IgG mAb (phage library derived)	R&D	(138–140)
RAC18	Ricin toxin from *Ricinus communis*	Ricin A chain	B	Murine mAb	R&D	(141)
c4C13	Ricin toxin from *R communis*	Ricin A chain	B	Chimeric mouse/human mAb	R&D	(142)
43RCA	Ricin toxin from *R communis*	Ricin A chain	B	scFv antibody fragment	R&D	(143)
HuMAb-154	SEB	SEB	B	Human IgG1	R&D	(144)
FL9, FL10	SEB	SEB	B	Human IgG mAbs (phage library derived)	R&D	(145)
Shigamabs cαStx1 and cαStx2v (Thallion Pharmaceuticals, Inc, Montreal, Canada)	Food- and water-borne pathogens	cαStx1 and cαStx2 Shiga toxins	B	Chimeric mouse/human mAb	Phase 2	NCT01252199

*National Institute of Allergy and Infectious Diseases category A, B, and C priority pathogens. (See https://www.niaid.nih.gov/research/emerging-infectious-diseases-pathogens for a breakdown of the classification of these biothreat categories.)

HBAT: heptavalent botulinum antitoxin; IgG: immunoglobulin G; IND: investigational new drug; mAb: monoclonal antibody; R&D: research and development; SEB: staphylococcus enterotoxin B

Data sources (as per chapter reference list): (137) Centers for Disease Control and Prevention. Investigational heptavalent botulinum antitoxin (HBAT) to replace licensed botulinum antitoxin AB and investigational botulinum antitoxin E. *MMWR Morb Mortal Wkly Rep*. 2010;59:299. (138) Avril A, Miethe S, Popoff MR, et al. Isolation of nanomolar scFvs of non-human primate origin, cross-neutralizing botulinum neurotoxins A1 and A2 by targeting their heavy chain. *BMC Biotechnol*. 2015;15:86. (139) Miethe S, Rasetti-Escargueil C, Liu Y, et al. Development of neutralizing scFv-Fc against botulinum neurotoxin A light chain from a macaque immune library. *Mabs*. 2014;6:446–459. (140) Rasetti-Escargueil C, Avril A, Chahboun S, et al. Development of human-like scFv-Fc antibodies neutralizing Botulinum toxin serotype B. *Mabs*. 2015;7:1161–1177. (141) Pratt TS, Pincus SH, Hale ML, Moreira AL, Roy CJ, Tchou-Wong KM. Oropharyngeal aspiration of ricin as a lung challenge model for evaluation of the therapeutic index of antibodies against ricin A-chain for post-exposure treatment. *Exp Lung Res*. 2007;33:459–481. (142) Guo JW, Shen BF, Feng JN, Sun YX, Yu M, Hu MR. A novel neutralizing monoclonal antibody against cell-binding polypeptide of ricin. *Hybridoma (Larchmt)*. 2005;24:263–266. (143) Pelat T, Hust M, Hale M, Lefranc MP, Dübel S, Thullier P. Isolation of a human-like antibody fragment (scFv) that neutralizes ricin biological activity. *BMC Biotechnol*. 2009;9:60. (144) Drozdowski B, Zhou Y, Kline B, et al. Generation and characterization of high affinity human monoclonal antibodies that neutralize staphylococcal enterotoxin B. *J Immune Based Ther Vaccines*. 2010;8:9. (145) Larkin EA, Stiles BG, Ulrich RG. Inhibition of toxic shock by human monoclonal antibodies against staphylococcal enterotoxin B. *PLoS One*. 2010;5:e13253.

product because rapid diagnostic determination of the toxin may not be available at the time of intoxication.

Staphylococcal enterotoxin B (SEB), one of multiple virulence factors of the gram-positive bacterium *Staphylococcus aureus*, is an extremely antigenic toxin in a family of many staphylococcal enterotoxins. Contact with naturally occurring *S aureus* can occur with these bacteria as they grow on the skin, mucosal surface, and can be found in food items. Previous proof-of-concept studies have demonstrated protection against toxic shock syndrome associated with staphylococcal enterotoxins utilizing human-derived IgG.[155] Currently, only a few mAbs targeting SEB toxins have been tested, and these used mouse models of protection. Derived from a human phage-display library, the first two mAbs, FL9 and FL10, demonstrated protection when premixed with the toxin.[145] HuMAb-154, a human antibody derived from hybridoma technology, demonstrated partial protection and delayed time to death when administered following increasing lethal doses of intraperitoneally administered SEB.[144] The human origin of these three antibodies supplies the advantage of reduced immunogenicity when intended for human use. However, these antibodies have yet to be used in a primate model or against aerosol exposure, experiments necessary to establish efficacy of the therapeutic potential against SEB for biodefense.

One of the main issues concerning toxin therapy using mAbs is treatment beyond 24 hours postexposure. Antitoxin antibodies are most effective when given early after exposure, and these timelines can be restrictive given the time it takes to detect and identify the toxin of interest. Although anti-ricin antibodies, when administered effectively, clear all toxins, antitoxin antibodies inhibit the activity of the toxin only, and not the pathogen itself (ie, neutralizing BoNT versus treating *C botulinum*). Thus, in the case of an infection, a treatment plan should be developed to eliminate both the toxin and the pathogen responsible for its production.

Antibacterial Monoclonal Antibodies

Many bacterial agents produce toxins. In addition to using mAbs against these bacterial toxins, mAb prophylaxis and therapy has targeted components on the surface of the bacterium, as with anthrax.[156] As early as 1890, antibacterial serum therapy proved successful, with the application of immune serum against diphtheria and tetanus developed by Emil von Behring, a German military doctor, who received a Nobel Prize for the initial development of pAbs.[132] With the germ theory growing more widely accepted throughout the 1900s, the first antibiotics were concurrently developed and often overshadowed the use of serum and antibody therapy. However, an increase in bacterial resistance has been observed across all existing antibiotic classes highlighting the need for the identification of new therapeutic options.[157] The early success of antibody-based therapies starting with polyclonal formulations and now more recent mAbs may constitute a therapeutic class capable to fill therapeutic gaps or even augment existing current therapies. Given the success of mAbs against cancer inflammatory and autoimmune diseases, it is surprising that more anti-infective mAb treatments have failed to make it to market. The primary factor inhibiting the progress of antibacterial mAbs is their lack of efficacy in animal studies and clinical testing, despite promising preclinical data.[156] (See Table 28-3[157–163] for more details.)

Anthrax, from *B anthracis*, is an agent that has been previously weaponized by state-sponsors and more recently used in an intentional dissemination of spores in the United States in 2001.[164] Early development of human antibody-based therapeutics against anthrax began after earlier antibodies of animal origin increased the therapeutic window of the disease and also shortened the duration of antibiotic treatment. Anthrax-specific antibody-based therapeutics act by a variety of mechanisms, either by targeting the capsule or by neutralizing the toxins that treat or augment therapy.[156] These antibodies represent the most advanced biodefense-related therapeutics, with the only two biodefense class specific mAbs, approved, and several others advancing through preclinical and clinical trials. These represent some of the most widely studied antibodies in the biodefense arena, with multiple mechanisms of action identified. Raxibacumab and Anthim (Elusys Therapeutics, Pine Brook, NJ) act by inhibiting PA-receptor interactions. Murine-derived antibodies 7.5G and 48.3 inhibit PA cleavage by furin, but thus far they have only been tested in murine models.[165,166] Thravixa (previously AVP-21D9; Emergent BioSolutions), currently in phase I clinical trial, inhibits PA heptamerization. Although no mAbs that directly inhibit the PA-LF/EF complex interaction are currently undergoing clinical evaluation, the potential for therapies has been demonstrated by human mAbs IZNLF, SS87, Fab A8, and 2LF, as well as the cross-reactive mAb H10. Other mechanisms have been shown by the chimeric chimpanzee/human mAbs LF10E and LF11H, which act by inhibiting endocytosis and translocation. However, LF10E and LF11H have only been tested in rodent models. Within all of these second generation therapeutics to anthrax, the specific mechanisms are expected to increase the therapeutic window, decrease length of treatment and assist in overcoming antibiotic resistance.

TABLE 28-3

ADVANCEMENTS OF TREATMENTS FOR BIODEFENSE BACTERIAL AGENTS UTILIZING ANTIBODY-BASED THERAPEUTICS

International Nonproprietary Name, Product Name, or Code	Targeted Agent	Targeted Antigen	Category*	Antibody Type (Isolation)	Approval, Clinical Phase, or Stage	US Patent or Reference No.
Raxibacumab/ABthrax (GlaxoSmithKline, Brentford, UK)	*Bacillus anthracis* (anthrax)	Anti-PA	A	Recombinant IgG1 mAb (naïve library)	Approved (2012)	601351
Valortim (Bristol-Myers Squibb, New York, NY)/MAb-1303	*B anthracis* (anthrax)	Anti-PA	A	Human mAb (transgenic mice)	Phase 1	7456264
Anthim Elusys Therapeutics (Elusys Therapeutics, Pine Brook, NJ)/ETI-204	*B anthracis* (anthrax)	Anti-PA	A	Chimeric deimmunized mAb (murine origin)	Phase 1	7446182
Thravixa (Emergent BioSolutions, Rockville, MD)/AVP-21D9	*B anthracis* (anthrax)	Anti-PA	A	Human mAb (hybridoma)	Phase 1	7438909, 7442373
Anthriving (Emergent BioSolutions)/AIG	*B anthracis* (anthrax)	Anti-PA	A	Polyclonal antibody from AVA human plasma	Phase 1/2	N/A
mAb 7.3 (plague)	*Yersinia pestis*	LcrV	A	Murine mAb (hybridoma)	R&D	(157)
m252/m252/m254 (plague)	*Y pestis*	F1/LcrV	A	Recombinant human mAb (naïve library)	R&D	(158)
mAb 3	*Francisella tularensis* (tularemia)	LPS	A	Murine IgG2a	R&D	(159)
6B3	*Brucella* species (brucellosis)	*B melitensis* LPS	B	Murine IgG3	R&D	(160)
2C8	*Brucella* species (brucellosis)	*B abortus* LPS	B	Murine IgG3	R&D	(160)
Ps6F6	*Burkholderia pseudomallei* (melioidosis)	Exopolysaccharide	B	Murine IgG3	R&D	(161)
1G2-1D3	*B mallei* (glanders)	LPS	B	Murine IgG2a	R&D	(162, 163)

*National Institute of Allergy and Infectious Diseases category A, B, and C priority pathogens.

AIG: anthrax immune globulin; AVA: Anthrax Vaccine Absorbed; IgG: immunoglobulin G; LPS: lipopolysaccharide; mAb: monoclonal antibody; N/A: not applicable; PA: protective antigen; R&D: research and development; IgG: immunoglobulin G

Data sources (as per chapter reference list): (157) Hughes JM. Preserving the lifesaving power of antimicrobial agents. *JAMA*. 2011;305:1027–1028. (158) Hill J, Copse C, Leary S, Stagg AJ, Williamson ED, Titball RW. Synergistic protection of mice against plague with monoclonal antibodies specific for the F1 and V antigens of *Yersinia pestis*. *Infect Immun*. 2003;71:2234–2238. (159) Xiao X, Zhu Z, Dankmeyer JL, et al. Human anti-plague monoclonal antibodies protect mice from *Yersinia pestis* in a bubonic plague model. *PLoS One*. 2010;5:e13047. (160) Lu Z, Roche MI, Hui JH, et al. Generation and characterization of hybridoma antibodies for immunotherapy of tularemia. *Immunol Lett*. 2007;112:92–103. (161) Laurent TC, Mertens P, Dierick JF, Letesson JJ, Lambert C, De Bolle X. Functional, molecular and structural characterisation of five anti-Brucella LPS mAb. *Mol Immunol*. 2004;40:1237–1247. (162) Bottex C, Gauthier YP, Hagen RM, et al. Attempted passive prophylaxis with a monoclonal anti-*Burkholderia pseudomallei* exopolysaccharide antibody in a murine model of melioidosis. *Immunopharmacol Immunotoxicol*. 2005;27:565–583. (163) Trevino SR, Permenter AR, England MJ, et al. Monoclonal antibodies passively protect BALB/c mice against *Burkholderia mallei* aerosol challenge. *Infect Immun*. 2006;74:1958–1961.

Bubonic, septicemic, and pneumonic plague are all caused by the gram-negative bacterium *Y pestis. Y pestis* contains two dominant targets for antibody-based therapeutics: (1) the surface polymer F1 and (2) the surface protein LcrV (as previously described in Plague, Chapter 10 in this volume). Early proof-of-concept studies using the murine anti-LcrV mAb 7.3 demonstrated complete protection against an aerosolized *Y pestis* challenge.[158] Other murine antibodies demonstrated similar protection, with mAb 7.3 and F1-04-A-G1 providing protection when administered as an aerosol cocktail prior to aerosolized challenge.[167] Naïve libraries have been used to produce the recombinant mAbs m252, m253, and m254. When used in a cocktail, these antibodies demonstrate synergistic protection, but they have been tested in murine models only and in the absence of antibiotics.[159] Because antibiotics are generally the first line of treatment, an understanding of the combined efficacy of antibody therapies and antibiotics is currently lacking in the antibody-based treatment of plague.

F tularensis is extremely virulent and difficult to identify with serological tests. Although *F tularensis* is susceptible to current antibiotics, an effective antibody-based therapeutic may be necessary because the various strains use different virulence mechanisms. Although these differences may explain the difficulty in obtaining an efficacious therapeutic, they also highlight the unique nature of each strain, which could be treated individually by using specific antibodies. The potential for effective antibody therapy against *F tularensis* in an aerosolized challenge model was first demonstrated by serum transfer of *F tularensis* LVS, which increased the mean time to death against the SchuS4 challenge strain.[168] The current antibody-based therapeutic options for *F tularensis* are of murine origin and have only been tested in rodent models.[160,169] Additional research and development will be needed in these areas to advance an effective biologic for clinical application.

The most successful utilization of antibacterial mAbs to date targeted the toxins of these organisms. These mAbs often possess extremely high affinity to their toxin targets, binding to and clearing the damaging toxins while allowing time for the host's immune response to clear the underlying bacterial infection. In contrast to toxin-exclusive neutralization, clearance mechanisms play a critical role in the therapeutic efficacy of these antibodies. In addition to the Fc-mediated clearance, it has been shown that antibodies can paradoxically increase the cytotoxic effects of these agents, as was seen in the enhancement of anthrax lethal toxin.[170] This complex interaction between the requirement for antibody-mediated clearance and the potential for negative antibody-dependent enhancement has also been recently reported for virus-specific antibody therapeutics.[171]

Antiviral Monoclonal Antibodies

Historically, vaccination has been the primary means of providing any medical countermeasure against viruses with bioterrorism agent potential. Following the eradication of smallpox, outbreaks of biodefense-related viruses in populations, with the exception of yellow fever, have been relatively infrequent. Several of these agents remain poorly understood due to their sporadic occurrence or orphan nature and limitations in resources or facilities capable of researching these viral diseases. The high mutation rate and poor understanding of many of these viruses account for the therapeutic gaps in protection, while the high infectivity and mortality make these an optimal bioweapon. For many viruses considered to have potential for use as biological weapons, including viral hemorrhagic fevers (VHFs) (eg, Ebola virus, Sudan virus, and Marburg virus) and *Togaviridae* encephalitides (eg, VEEV, eastern (EEEV), and western (WEEV) equine encephalitis viruses), only supportive therapy exists following exposure. Ribavirin, a nonimmunosuppressive nucleoside-analogue with broadly protective antiviral properties, has demonstrated protection only against the VHF Lassa virus and is available only for compassionate use under an IND application.[172] Additional studies have indicated that the use of ribavirin would be effective against other arenaviruses as well as Bunyaviruses (see Alphaviruses, Chapter 20 in this volume). Beyond ribavirin, few other drug options exist, leaving only supportive care in many cases.

The successful application of therapeutic antibodies has been demonstrated previously. The National Institute of Allergy and Infectious Diseases (NIAID) categorizes biodefense agents based on several factors including, but not limited to, the agent's pathogenesis, dissemination, available treatments, as well as the agent's mortality. Category A agents are considered the most dangerous, based on ease of dissemination, high mortality, public health impact, and absence of available therapeutics, with Categories B and C following to a lesser extent in one or all of these areas. This chapter has focused primarily on Categories A and B agents; however, two category C agents, Hendra virus and Nipah virus, have well-characterized therapeutic mAbs for postexposure treatment. (See Table 28-4[173–183] for more details.) The human mAb m102.4 demonstrated protection against Nipah in the ferret model[184] and Hendra in a nonhuman primate (NHP) model[183] as a postexposure therapeutic as late as 7 to 8 days after infection.

TABLE 28-4

ADVANCEMENTS OF TREATMENTS APPLIED ON BIODEFENSE VIRAL AGENTS UTILIZING ANTIBODY-BASED THERAPEUTICS

International Nonproprietary Name, Product Name, or Code	Targeted Agent	Targeted Antigen	Category*	Antibody Type (Isolation)	Approval, Clinical Phase, or Stage	Patent, Trial Number, or Reference No.
ZMapp (13C6, 2G4, 4G7)	Filovirus (Ebola)	Ebola Zaire GP	A	Cocktail of three recombinant human mAbs	Phase 1/2, utilized EUA	NCT02389192, NCT02363322 (173)
ZMab (1H3, 2G4, 4G7)	Filovirus (Ebola)	Ebola Zaire GP	A	Cocktail of three recombinant human mAbs	Utilized EUA	(174)
MB-003 (13C6, 13F6, 6D8)	Filovirus (Ebola)	Ebola Zaire GP	A	Cocktail of three recombinant human mAbs	Utilized EUA	(175)
8AH8AL	Variola major (smallpox)	B5	A	Chimeric chimpanzee/ human mAb	R&D	(176)
6C	Variola major (smallpox)	A33	A	Chimeric chimpanzee/ human mAb	R&D	(177)
hV26 / h101	Variola major (smallpox)	H3/B5	A	Human mAbs (transgenic mice)	R&D	(178)
hB5RmAb	Variola major (smallpox)	B5	A	Human mAb (derived from rat)	R&D	USP 7811568
Vaccinia immune globulin	Variola major (smallpox)	Whole antigen	A	16.5% IgG from vaccinia virus individuals	Approved for vaccine SE	(179)
Hu1A3B-7	VEE	E2 GP	B	Humanized mAb IgG1 (derived from mouse)	R&D	(180)
Hu1A4A-IgG1-2A	VEE	E2 GP	B	Humanized mAb IgG1 (derived from mouse)	R&D	(181)
ToR67-3B4	VEE	E1 GP	B	scFv-Fc fusion protein (NHP phage display)	R&D	(182)
m102.4	Nipah / Hendra	GP G	C	Recombinant IgG1 mAb	Preclinical development	(183)

*National Institute of Allergy and Infectious Diseases category A, B, and C priority pathogens.

EUA: Food and Drug Administration Emergency Use Authorization; GP: glycoprotein; mAb: monoclonal antibody; NHP: nonhuman primate; R&D: research and development; SE: side effects; USP: US Pharmacopeia; VEE: Venezuelan equine encephalitis

Data sources (as per chapter reference list): (173) Qiu X, Wong G2, Audet J, et al. Reversion of advanced Ebola virus disease in nonhuman primates with ZMapp. *Nature*. 2014;514:47–53. (174) Qiu X, Audet J, Wong G, et al. Sustained protection against Ebola virus infection following treatment of infected nonhuman primates with ZMAb. *Sci Rep*. 2013;3:3365. (175) Pettitt J, Zeitlin L, Kim do H, et al. Therapeutic intervention of Ebola virus infection in rhesus macaques with the MB-003 monoclonal antibody cocktail. *Sci Transl Med*. 2013;5:199ra113. (176) Chen Z, Earl P, Americo J, et al. Chimpanzee/human mAbs to vaccinia virus B5 protein neutralize vaccinia and smallpox viruses and protect mice against vaccinia virus. *Proc Natl Acad Sci U S A*. 2006;103:1882–1887. (177) Chen Z, Earl P, Americo J, et al. Characterization of chimpanzee/human monoclonal antibodies to vaccinia virus A33 glycoprotein and its variola virus homolog in vitro and in a vaccinia virus mouse protection model. *J Virol*. 2007;81:8989–8995. (178) McCausland MM, Benhnia MR, Crickard L, et al. Combination therapy of vaccinia virus infection with human anti-H3 and anti-B5 monoclonal antibodies in a small animal model. *Antivir Ther*. 2010;15:661–675. (179) Hopkins RJ, Lane JM. Clinical efficacy of intramuscular vaccinia immune globulin: a literature review. *Clin Infect Dis*. 2004;39:819–826. (180) Goodchild SA, O'Brien LM, Steven J, et al. A humanised murine monoclonal antibody with broad serogroup specificity protects mice from challenge with Venezuelan equine encephalitis virus. *Antiviral Res*. 2011;90:1–8. (181) Hu WG, Phelps AL, Jager S, et al. A recombinant humanized monoclonal antibody completely protects mice against lethal challenge with Venezuelan equine encephalitis virus. *Vaccine*. 2010;28:5558–5564. (182) Rulker T, Voss L, Thullier P, et al. Isolation and characterisation of a human-like antibody fragment (scFv) that inactivates VEEV in vitro and in vivo. *PLoS One*. 2012;7:e37242. (183) Geisbert TW, Mire CE2, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. *Sci Transl Med*. 2014;6:242ra82.

Before the development of mAb-based therapeutics, passive antibody therapy had been the only option for treating VHFs, but these efforts yielded mixed results.[185] Despite previous successful results, concerns about the transmissibility of blood-borne pathogens from donor sera to recipients remain. In many cases, the diagnostic screening capabilities needed to confirm that a sample is pathogen-free before administration within the therapeutic window are not present. However, passive immunotherapy has provided the initial necessary evidence that therapeutic antibodies can be an effective preexposure or postexposure therapeutic.[185]

Despite the promise shown by passive immunotherapy, early negative experimental evidence limited interest in the application of antibody-based therapeutics against VHFs, specifically the Filoviruses. Previous passive transfer studies against Lassa virus demonstrated effectiveness if given early in the course of infection.[186] During the 1995 Kikwit Ebola outbreak, crude blood transfusions were used as an immunotherapy, resulting in a death rate of one in eight, in comparison to an 80% mortality rate for those untreated.[187] Studies using hyperimmunized equine serum against Ebola virus administered to macaques demonstrated delayed time to death but no change in the survival rate.[188] The first attempt at protecting against Ebola virus used KZ52, a human antiglycoprotein mAb.[189] KZ52 effectively neutralized Ebola virus in plaque assays and, when passively administered, protected guinea pigs,[190] but it failed to protect or affect disease progression when given to NHPs.[191]

One explanation for the failures of passive immunization of pAbs or single mAbs to protect against Filoviruses is that this therapy controls the viral burden initially, but once depleted, the virus overwhelms the system. However, in 2012 passively transferred species-matched pAbs were found to provide complete protection in an NHP model, demonstrating the capability of antibody-based therapies against Filoviruses.[192] Recent oligoclonal, or cocktail, mixtures of antibodies that target multiple epitopes of the virus—such as MB-003,[175] ZMAb,[174] and ZMapp[173]—have demonstrated protection in infected NHPs. Furthermore, ZMapp was administered under emergency use authorization to two healthcare providers infected with Ebola during the 2014 West Africa outbreak who subsequently survived the infection.[3] Similar to vaccines that provide humoral immunity, such as VEEV replicon particles,[193] ZMAb provided complete sustained protection 10 weeks posttreatment upon re-challenge in NHPs.[174] These studies, taken together with published data specific to the correlates of Filovirus protection,[194] provide evidence of the ability of antibody therapy to provide protection until the host's humoral immune system initiates a response.

Historically, smallpox is one of the most concerning of all biothreat agents. Even with a successful vaccination program, smallpox remains a potential biological weapon because of the large nonvaccinated population. Other than vaccination, the only approved therapeutic is vaccinia immune globulin, a pAb extracted from vaccinated humans. Despite its limited potency against the disease,[179,195] this product is approved to reduce the potential side effects of the vaccine and has been shown to reduce morbidity and mortality associated with smallpox.[179] Several products have been developed using animal-based isolation techniques, although none are in clinical trials. Given the restricted access to the smallpox virus, new antibody-based therapeutics have been compared with vaccinia immune globulin using the vaccine strain for challenge. Several products have emerged in recent years from phage-display technology using chimpanzees,[176,177] transgenic mAbs,[178] and humanized rat mAbs[196]; mAbs developed against smallpox have been isolated from either vaccinated or infected animals. The humanized mAb hB5RmAb, whose parental antibody was isolated from a rat, and 8AH8AL, a chimeric chimpanzee/human recombinant antibody (rAb) derived from phage display, are both directed against the B5 surface protein of the extracellular enveloped virions.[176,196] Additionally, two human mAbs, hV26 and h101, were isolated from transgenic mice and bind to the H3 protein found at the surface of mature virus and to the B5 protein, respectively.[178] All of these antibodies elicited protection in various mouse models; however, none have been tested in NHP models of disease, as recommended by the World Health Organization.

Several viruses of the Alphavirus genus cause encephalitis, and of these, VEEV and EEEV are classified as category B select agents. Early animal studies using passive transfer of neutralizing antisera and mAbs demonstrated this therapy's protection against Alphaviruses.[197–199] Early proof-of-concept studies have shown human constructs and recombinant mAbs to be successful in providing protection in mice.[181,200,201] The humanized mAb Hu1A3B-7 (IgG1 isotype) binds the E2 glycoprotein and is broadly specific to VEEV subtypes, neutralizing type IAB (Trinidad donkey or TrD), type II (Fe37c), and type IIIA (Mucambo BeAn8) in vitro. Hu1A3B-7 administered intraperitoneally 24 hours postchallenge provided complete protection against subcutaneous challenge of 100 times the median lethal dose (LD_{50}) of VEEV TrD, as well as 90% protection when challenged against 100 LD_{50} by aerosol.[200] Hy4 is a humanized antibody that binds to the VEEV E2

protein. When administered intraperitoneally 1 or 24 hours postchallenge with VEEV TrD, just 10 μg elicited 90% and 75% protection. Administration of 500 μg of Hy4 24 hours prechallenge provided 80% protection against intranasal challenge of 1,350 plaque-forming units.[201] The mAb Hu1A4AIgG1-2A, humanized from murine 1A4A, binds to the E2 glycoprotein of VEEV with high affinity. The Hu1A4AIgGIgG1-2A provided both prophylactic and therapeutic protection against subcutaneous administration of VEEV TrD.[181] The recently developed ToR67-3B4, an NHP phage-display derived scFv-Fc fusion antibody directed to the E1 protein, represents one example of the next generation of constructs providing protection against Alphaviruses.[182] This antibody provided 83% protection against an aerosol challenge of VEEV TrD, with limited protection at later times in the mouse model.[182] The antibodies that protected against VEEV were only tested in mice. None of the above antibodies have been tested in larger models; any potential therapeutic for biodefense would need to be used in models currently under development.[202]

Unlike other Department of Defense biothreat agents, Alphaviruses have a neuroinvasive component, which limits the effectiveness of antibody-based therapeutics due to their inability to cross the blood–brain barrier (BBB). Although active infection can inhibit the BBB's ability to filter, allowing some immune therapeutics to pass through late during infection, new therapeutic designs with the ability to cross the BBB would facilitate treatments. Developers of the next generation of Alphavirus-based antibodies should endeavor to design antibodies with a capability to cross the BBB. One possible format for these therapeutics would be bispecific antibodies (see Figure 28-3). Several antibodies have been developed for diseases and conditions unrelated to biodefense that could serve as a model for the development of BBB-crossing therapeutic mAbs against Alphaviruses.[203] Other antibody formats developed outside of biodefense include complete trifunctional chimeric IgGs and the scFv-scFv constructs.[204] While a broadly reactive mAb is ideal, the high mutation frequency of Alphaviruses and other RNA viruses raises concern about the potential emergence of resistant strains.[147] A monoclonal therapeutic will most likely require an oligoclonal product, or cocktail of several antibodies, each broadly reactive and capable individually of neutralizing the virus.

Any successful viral infection requires viral particles to be released into extracellular space; however, with some biodefense-related viruses, such as variola major, that require just one inhaled particle-forming unit to initiate an infection, the utility of circulating antibodies becomes limited.[205] Viral latency has been one of the major challenges in developing effective antibody-based therapeutics against HIV.[206] Furthermore, as demonstrated by the brief protection windows described above, many biodefense-related viral pathogens require rapid identification and antibody administration within the first 48 to 72 hours to be effective. This is typically the case for Alphaviruses that cross the BBB and Filoviruses that rapidly overwhelm the immune system with high viral load.

In addition to rapid diagnosis, having a greater understanding of biodefense agents and their pathogenesis in host model systems will greatly aid in the ability to more quickly identify and develop therapeutics. For example, the identification of Alphavirus glycoprotein glycosylation sites and successful production of recombinant glycoproteins would allow for rapid screening of target antibodies. Similarly, the development of an animal model for Crimean-Congo hemorrhagic fever would supply an in vivo model system to test therapeutic efficacy. These are just two examples of basic tools and knowledge that could significantly enhance the productivity and efficacy of vaccine and therapeutic development efforts. To achieve the greatest success, future work should therefore focus on the development of appropriate reagent material and model development in parallel with the programmatic development of therapeutic and vaccine candidates.

SUMMARY

Vaccines and antibody-based therapeutics are some of the greatest achievements in global health improvement in previous centuries. Despite these advances, biodefense vaccines and treatments remain scarce, and there is a great need to define future requirements specific to biodefense vaccines and antibodies. Enormous advances have been made in the fields of vaccines and antibody-based medical countermeasures, and many creative strategies have been developed that may address the current needs; however, the barriers between an idea or concept and a product are vast, and costs to develop one product can surmount $100 million.

The first challenges in the future development of vaccine or immunotherapy medical countermeasures will be how to prioritize the funding for an ever-growing pipeline of products and whether to develop vaccines or antibodies (or a combination of both). Within the limited funding environment of infectious diseases and toxins, focus should be agent-specific for the development of specific vaccines and antibody-based therapeutics. One option would be to focus vaccine

development primarily on communicable diseases, for which the threat of epidemic outbreaks is a primary concern. Then, noncommunicable diseases and toxins, or those diseases that are poorly transmitted, could be addressed by development of antibody-based therapeutics. Alternative approaches could then be used to augment the initial round of medical countermeasures.

New vaccine and therapeutic development should not only be aligned with the relative ease in obtaining many of the more historical biological agents (eg, ricin or anthrax) as a determining factor, but also should be aligned to the categorization of the agents and availability or absence of availability of effective vaccines and/or therapeutics for the higher category agents. The Amerithrax anthrax attacks highlight the panic and fear that can quickly disrupt public, commercial, and governmental activities with localized instances of infection. This public fear perception is the principal reason why Filovirus infections, specifically Ebola virus, attract so much attention in contrast to other infectious diseases that kill far more people annually from ongoing epidemic outbreaks (eg, influenza virus). In the United States, a new strategy using a "whole-government" approach has been implemented by the National Interagency Confederation for Biological Research to coordinate efforts for the development of medical countermeasures.[207] Internationally, NATO (North Atlantic Treaty Organization) panels, cooperative agreements, and basic science partnerships are also being used to reduce the overall cost and impact of incorporating more novel means for developing countermeasures on tighter budgets. Involvement in these cooperative strategies should be leveraged at the interagency and international level as a means of cost reduction, as well as a diversification of expertise as the community searches for the next generation of medical countermeasures.

Biodefense vaccines may also face several more general issues of approval and licensure, much like common vaccines, but with the added requirements of approval under the Animal Rule because many of these diseases do not exist for an adequate Phase II/III study. Vaccine approval, even in the military, can be challenging, as illustrated by the anthrax vaccine campaign that was interrupted by the Department of Defense in 1999.[208] Vaccine safety standards have become more stringent over the last few decades, and biodefense vaccines must be held to these standards. Whereas confidence in vaccine efficacy has also improved, the task of ensuring vaccine safety can be daunting, as exemplified in spring 2010 by the increased risk of narcolepsy and catalepsy observed in patients in several countries after H1N1 vaccination.[209] Another concept that may need to be integrated into biodefense vaccines is individualized medicine. Next-generation vaccines may be targeted to specific subpopulations according to their HLA genotypes or the capacity of their immune system to mount an appropriate immune response.

The next generation of antibody development should focus on the mechanism of how antibodies enter into or influence a cellular environment. For example, "transbodies" are cell-permeable antibodies made through conjugation of an antibody to a ligand to facilitate entry of the antibody into the cell or to inhibit a specific function, as with immunotoxins. Conversely, "intrabodies" are antibodies developed to achieve intracellular expression using the application of recombinant DNA technology.[210]

Even with these novel means of antibody delivery and action, target identification remains one of the largest challenges in developing the next effective antibody therapeutics. Secondarily, antigen-binding specificity and access are other points to consider because antibodies are highly specific to the target, and corresponding antigens from different species, such as NHPs or rodents, are dependent on the antigen alignment between species. In many cases, the critical epitopes targeted by vaccines and therapeutics are conserved and additional mitigation has been achieved by the use of oligoclonals, or cocktails of antibodies. These critical epitopes are of particular concern for both vaccines and therapeutic antibodies because biodefense-related treatments often require special use of the FDA Animal Rule for advancement through clinical trials.

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