Antibody-Based Defense Strategies against Biological Weapons

An immune-based approach against infectious diseases developed near the end of the 19th century brings renewed opportunities at the start of the 21st century. Arturo Casadevall

Antibody-based therapies that were developed at the end of the 19th century provided the first effective therapies against many infectious diseases. By the 1930s, passive antibody therapies were routinely used in patients with pneumococcal pneumonia, meningococcal meningitis, pertussis, scarlet fever, and some toxin-mediated diseases such as diphtheria and tetanus. In the preantibiotic era, therapeutic antibodies were typically produced in large animals as immune sera because they were a more practical source of materials than were human sources. Hence, the early forms of antibody therapy were known as serum therapy.

Despite unquestioned efficacy in certain patient groups, antibody-based therapy had significant drawbacks, including toxicity in the form of allergic reactions to animal proteins, high cost, a need for a specific diagnosis prior to therapy, dose standardization difficulties, and loss of efficacy when treatment was delayed. In contrast to serum therapy, antimicrobial drugs are effective against a broad variety of microbes and may be administered empirically. Consequently, serum therapy was largely abandoned in the 1940s because antimicrobial chemotherapy and antibiotics proved less toxic, cheaper, and more convenient to administer.

Paradoxically, the decline and abandonment of serum therapy coincided with major advances in the understanding of antibody structure and the development of improved techniques for purifying immunoglobulins, each of which might have helped overcome some of the drawbacks of antibody therapy had they been applied to solving clinical use problems.

A Resurgence of Antibody-Based Therapies in Medical Practice

Serum therapy did not really disappear after sulfonamides and penicillin were introduced into clinical practice. Instead, serum therapy remained useful in niche areas where other options remained unavailable, including for treatment of venom- and toxin-mediated diseases. Moreover, vaccinia immune globulin was useful for treating the relatively rare but sometimes serious side effects experienced by those vaccinated to protect against smallpox.

Beginning in the 1960s, there was renewed interest in developing antibody therapies for viral diseases. Thus, separate immune globulin preparations were introduced into clinical practice for use in preventing and treating diseases caused by the hepatitis B, rabies, and respiratory syncytial viruses. In contrast to older formulations, which relied largely on animal sera, these specific immune globulins contained antibodies derived from immunized human donors and, consequently, had minimal side effects. Although human-specific globulins lack toxicities associated with animal sera, they are expensive, available in limited supply, and can transmit diseases.
Arturo Casadevall believes much of his education about life came from working in a Queens, N.Y., fast-food restaurant. Starting at age 16, he spent four years during high school and college on the night shift, rotating from one duty to another—although his strongest memories are of prolonged standing at the french-fry station.

"Much of what I know I learned there," he says. "You learn discipline, responsibility, and, most importantly, how to work with others. Fast food places emphasize the value of teamwork and how the work of one individual is dependent on the whole team. I apply those lessons every day in managing a laboratory and clinical division."

Casadevall, 47, who came to the United States from Cuba in 1968 when he was 11, today heads the infectious diseases division at Montefiore Medical Center-Albert Einstein College of Medicine. He also serves as professor of medicine, microbiology, and immunology at the college, and is the Selma and Jacques Mitranzi professor of biomedical research.

"My life was your typical immigrant experience," he says, referring to his childhood. "Not much here that could account for how I did later in life. I discovered science early and loved it. In college, I did some research electives and discovered that I really liked it. Also, I have been greatly influenced by teachers and scientific mentors—I have been extremely lucky in this regard."

He received his B.A. degree in chemistry in 1979 from Queens College and his M.S., Ph.D., and M.D. degrees from New York University. His graduate thesis research was supervised by Loren Day in the field of physical biochemistry and focused on DNA-protein structures in filamentous bacteriophages. Afterwards, he completed residency training in internal medicine at Bellevue Hospital in New York City. This was in the late 1980s, when AIDS was cutting a deadly swath through the region—and he saw first hand the effects of the epidemic. The experience fueled his interest in infectious diseases research. He subsequently completed postdoctoral training with Matthew Scharff at Albert Einstein College of Medicine investigating the molecular genetics of the immune response to *Cryptococcus neoformans*.

"I am a physician who is also a basic researcher," Casadevall says. "I am interested in developing new therapies using the immune system and, in particular, antibody administration. I care because I have seen too much human suffering and would like to make a difference in some small way."

One of his lab’s major projects is to develop an antibody-based therapy for treating fungal infections. "In this regard, I was very influenced by my clinical experience where I saw people die despite antifungal therapy because drugs were insufficient to clear the infection in the setting of immunosuppression. So I guess you can say that my clinical experiences have greatly shaped what I do for a living in the laboratory."

Therapeutic antibodies already are making a comeback; they are now widely used in treating certain cancers, asthma, and rheumatoid conditions, according to Casadevall, who is happy to see their return. "The field of infectious diseases pioneered antibody use in the early 20th century, but then abandoned it in favor of antibiotics," he says. "Recently there is a lot of interest in using this therapy. It has some significant advantages: it should not select for resistance in nontargeted microbes; it is relatively safe and nontoxic; it is effective."

"The major problem is cost—these therapies are expensive. But I would argue that the current strategy of antibiotics is more expensive in the long run with the problems of resistance and superinfections," he adds.

Casadevall is married to a physician and they have a teenage son. His one major interest outside science is history. "I am an avid reader of historical books," he says. He also describes himself as an optimist, adding: "Science is natural for optimists."

**Marlene Cimons**

Marlene Cimons is a freelance writer who lives in Maryland.
The monoclonal antibody (mAb) era began in 1975 when hybridoma technology provided a means for producing substantial amounts of single antibodies having one isotype and specificity. In the past two decades, investigators who applied recombinant DNA technology to the challenges of generating and engineering such antibodies have produced a wealth of reagents with potential usefulness in treating infectious diseases. However, most of the recent interest in developing new antibody-based therapies has focused on oncology, allergy, and autoimmune diseases, with only one mAb currently licensed for treating an infectious disease. Even so, the aggregate experience from evaluating hundreds of mAbs, albeit for noninfectious diseases, should greatly facilitate comparable development of mAbs for antimicrobial applications. Indeed, the regulatory, industrial, and logistical infrastructure is now in place for developing useful antimicrobial, mAb-based reagents.

**Immunoglobulins and Standard Antimicrobial Drugs**

In the field of infectious diseases any discussion about the possibilities of antibody-based therapy inevitably involves a comparison to antimicrobial drugs. Of course, the fairness and relevance of such comparisons should be questioned, especially because combination antibiotic-antibody therapies often prove synergistic or at least additive.

Consider the intrinsic traits of these two classes of agents (see table). Antibodies are large, multifunctional molecules composed of two or more protein chains that act by binding microbial antigens to exert microbicidal effects either directly or by combining with other components of the immune system. The molecular weights of immunoglobulin G (IgG) and IgM are approximately 150,000 and 900,000 Da, respectively. In contrast, antimicrobial drugs typically are relatively small molecules that function by interfering with one or more critical steps in microbial physiology. Antibodies generally are pathogen-specific drugs, whereas antimicrobial drugs typically act against several or many types of microbes.

Thus, antibody therapies require a specific diagnosis, whereas antimicrobial therapy can be used empirically. Standard antimicrobial drugs are usually cleared from the body rapidly and consequently require frequent dosing to maintain active levels, whereas immunoglobulins have serum half-lives of up to 20 days, meaning that a single dose provides relatively long-lasting protection.

**Antibodies for Defense against Biological Weapons**

Passive antibody administration is currently the only means of conferring immediate immunity to a susceptible host—a property that makes immunoglobulins attractive for protecting us against biological weapons. These protective agents offer a sharp contrast to vaccines, which require weeks or months to elicit a protective response and also depend on the host immunological status to work effectively. Thus, infusing specific antibodies into someone who is otherwise vulnerable to a specific infectious agent leads to immediate resistance to the corresponding microbial pathogen. Moreover, because the serum half-life of human IgG can be 20 days, administering antibodies provides extended protection during which period other countermeasures may be put to use, including vaccination, environmental decontamination, and threat neutralization.

In the face of an attack, antibodies can be used prophylactically to prevent disease before, or therapeutically after, infection or toxin acquisition. One logistical complexity to antibody-based therapies is the need for their systemic administration. Because antibody-based therapies are usually administered intravenously, this approach would be impractical when large populations are attacked. However, antibodies also may be administered by injecting them into muscle tissue, making mass administration from self-injectable devices potentially feasible.

Another complication with antibodies is that they usually need to be kept cold to be stable. Hence, deploying an antibody-based strategy for defending against biological attacks would entail establishing, maintaining, and monitoring refrigerated immunoglobulin storage facilities. Fortunately, however, when kept refrigerated, immunoglobulins are remarkable stable proteins, and long-term storage should be possible. Furthermore, advances in antibody engineering could allow development of preparations with
longer shelf lives and perhaps even their storage as lyophilized proteins. Investments now to develop better means for making, using, and storing therapeutic antibodies could help to make their current high cost more palatable since they could retain activity for protection of future generations.

### Passive Antibody Strategies Present Additional Challenges

There are other challenges to face when anticipating widespread medical uses of passive antibodies. For example, they typically are most effective when given before an individual en-

<table>
<thead>
<tr>
<th>Comparison of immunoglobulin therapies and antibiotics</th>
<th>Immunoglobulin type</th>
<th>Antimicrobial drug</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parameter</strong></td>
<td><strong>Immune sera</strong></td>
<td><strong>mAb</strong></td>
<td></td>
</tr>
<tr>
<td>Activity</td>
<td>Low</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Administration logistics</td>
<td>Complex</td>
<td>Complex</td>
<td>Simple</td>
</tr>
<tr>
<td>Action</td>
<td>Microbicidal</td>
<td>Microbicidal</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Toxin neutralizing</td>
<td>Toxin neutralizing</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Immunomodulatory</td>
<td>Immunomodulatory</td>
<td></td>
</tr>
<tr>
<td>Cost</td>
<td>High</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Dosing</td>
<td>Infrequent</td>
<td>Infrequent</td>
<td>Frequent</td>
</tr>
<tr>
<td>Origin</td>
<td>Immune donors</td>
<td>Hybridoma, expression system</td>
<td>Immune sera is obtained from immunized animal or human donors</td>
</tr>
<tr>
<td>Resistance</td>
<td>Low</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Specificity</td>
<td>Narrow</td>
<td>Narrow</td>
<td>Broad</td>
</tr>
<tr>
<td>Toxicity</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Time to development</td>
<td>Short</td>
<td>Intermediate</td>
<td>Long</td>
</tr>
</tbody>
</table>

Immune sera has low specific activity because only a small fraction of the total protein is antibody active against the agent. Immunoglobulins require cold chain and systemic administration. In contrast, many antimicrobial drugs can be given orally and stored at ambient temperatures. Some immunoglobulins are directly microbicidal and others mediate action through immunomodulatory effects. The cost of immunoglobulins is higher because they are obtained from donors or tissue culture, require cold chain, and complex purification protocols. With serum half lives of up to 20 days immunoglobulins require infrequent dosing. Each agent can be defeated by engineered microbial resistance. In the case of mAbs vulnerability is high since because only one epitope is involved. Immune sera is less vulnerable because it usually contains antibodies of multiple specificities. Immunoglobulins are usually pathogen specific whereas most antimicrobial drugs have activity against multiple pathogens. Purified immunoglobulin preparations have low toxicity although there is always the concern of inadvertent contamination with microbial agents given that they originate from living cells. Immune sera can be generated relatively quickly. In the early 1900s serum for the treatment of meningococcal meningitis was develop during the course of an epidemic.
counters an infectious agent or toxin. During the preantibiotic era when passive antibodies were being used, patients and their doctors learned that efficacy declined if the antibodies were administered after the onset of symptoms. For example, serum therapy typically remained effective if taken during the first three days after the onset of symptoms; moreover, it was much more effective on the first day than on the third.

Although this loss in efficacy is not understood, it may be due to increasing numbers of microorganisms being present in the host or because the disease has progressed to a point where this type of therapy is no longer active. In this regard, antibody therapies are less effective than antimicrobial chemotherapy, which typically remains effective for several days after symptoms appear. Hence, rapid diagnostic tests will be needed when deploying antibody-based strategies.

Antigenic changes in microbes, whether natural or genetically engineered, can undermine the efficacy of antibody therapies. Indeed, mAbs to a single determinant are particularly vulnerable. Although several mAbs can be combined in a polyclonal treatment cocktail, their combined use introduces complexity, especially when evaluating such mixtures for safety and efficacy in a drug regulatory context.

Enhancing the Therapeutic Properties of Antibodies

Late-20th-century concepts in immunology and host defense mechanisms emphasized a division of labor for the immune system in which antibodies were believed to protect primarily against extracellular pathogens, parasites, and certain viruses, whereas cellular immunity was thought to protect mainly against intracellular pathogens, mycobacteria, fungi, and most other viruses.

However, learning that mAbs can protect against many types of microbes that supposedly were under the exclusive purview of cellular immunity challenged that once-orthodox view. For instance, humoral immunity was once considered to have little or no role in protecting against *Mycobacterium tuberculosis*, *Listeria monocytogenes*, *Cryptococcus neoformans*, and *Histoplasma capsulatum*. In recent years protective monoclonal antibodies have been made to each of these pathogens.

Moreover, antibodies are proving to be far more versatile natural reagents than once thought. Functions now attributed to such molecules include direct antimicrobial effects, toxin neutralization, viral neutralization, interference with microbial attachment, complement activation, and modulation of immune responses through activation of Fc receptors. This versatility suggests that for practically any pathogen, there should be an antibody that enhances host defenses.

Another example of this versatility is that antibody molecules may be modified to enhance their antimicrobial properties. For example, attaching nuclides to mAbs makes them directly microbicidal to bacteria and fungi. Furthermore, antiviral mAbs can be made cytotoxic by attaching toxins to them that kill virus-producing cells that express viral antigen on their surfaces. Thus, even nonprotective antibodies can be imbued with antimicrobial properties, enhancing their effectiveness as therapeutic agents.

Overcoming Basic Science, Clinical Hurdles

Although Behring and Kitasato described antibody-mediated immunity in 1890 and this topic was intensely studied during the first part of the 20th century, our understanding of this process remains rudimentary. The classical functions associated with antibody-mediated protection are opsonization, complement activation, toxin neutralization, viral neutralization, and antibody-directed cellular cytotoxicity. Meanwhile, recent additional functions ascribed to antibodies include modulation of inflammatory reon other immune components. Other poorly understood problems are the role of isotype in antibody-mediated protection and the specificity requirements for antibody efficacy. Moreover, because we cannot predict which antibodies will protect against which microbes, the development of antibody-based therapies remains a largely empirical endeavor.

Apart from these uncertainties, any rapid deployment of antibodies to defend against biological attacks is likely to proceed without results from clinical efficacy trials. The impossibility of conducting such trials implies that, for each
biological weapon to which antibodies are developed, we will need other means to understand the correlates of immunity, the mechanisms of antibody-mediated protection, and the amounts of antibody needed to ensure immunity.

Despite these hurdles, it is worth remembering that many effective anti-infective antibody therapies were developed early during the 20th century, well before much was understood about the nature of proteins, antigens, and immune mechanisms. The success of serum therapy a century ago and its current renaissance for treating neoplastic and inflammatory diseases provide encouragement and optimism about the validity of this strategy for protecting us against biological weapons. Nevertheless, we should also recognize that our best protection against these terrible weapons is a layered defense that includes not only antibodies but also simultaneous improvements in surveillance, diagnostic methods, vaccines, antimicrobial drugs, the infrastructure for the delivery of health care, and education.

SUGGESTED READING


