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History and Practice: Antibodies in Infectious Diseases

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ABSTRACT Antibodies and passive antibody therapy in the treatment of infectious diseases is the story of a treatment concept which dates back more than 120 years, to the 1890s, when the use of serum from immunized animals provided the first effective treatment options against infections with *Clostridium tetani* and *Corynebacterium diphtheriae*. However, after the discovery of penicillin by Fleming in 1928, and the subsequent introduction of the much cheaper and safer antibiotics in the 1930s, serum therapy was largely abandoned. However, the broad and general use of antibiotics in human and veterinary medicine has resulted in the development of multi-resistant strains of bacteria with limited to no response to existing treatments and the need for alternative treatment options. The combined specificity and flexibility of antibody-based treatments makes them very valuable tools for designing specific antibody treatments to infectious agents. These attributes have already caused a revolution in new antibody-based treatments in oncology and inflammatory diseases, with many approved products. However, only one monoclonal antibody, palivizumab, for the prevention and treatment of respiratory syncytial virus, is approved for infectious diseases. The high cost of monoclonal antibody therapies, the need for parallel development of diagnostics, and the relatively small markets are major barriers for their development in the presence of cheap antibiotics. It is time to take a new and revised look into the future to find appropriate niches in infectious diseases where new antibody-based treatments or combinations with existing antibiotics, could prove their value and serve as stepping stones for broader acceptance of the potential for and value of these treatments.

Antibodies in Infectious Diseases aims to inform, update, and inspire students, teachers, researchers, pharmaceutical developers, and health care professionals on the status of the development of antibody-based therapies for treating infectious diseases and the potential for these in times of growing antibiotic resistance to provide alternative treatment solutions to the currently used antibiotics and new treatments for infectious diseases where no proper treatments are available.

This introductory article will provide a historical perspective on the use of antibody-based therapies, followed by a high-level overview of what makes antibodies attractive tools for this purpose. This will include the pros and cons of such therapies compared to the use of antibiotics and the practical and strategic considerations involved in selecting the best format and development path for new antibody-based therapies targeting specific infectious agents. Then, examples of antibody-based therapies in the development of treatments for infectious diseases will be presented, and finally a look into the future will summarize the different aspects that will influence what the future might bring for this type of treatments for infectious diseases.

HISTORICAL PERSPECTIVE

Antibodies and the use of passive antibody therapy in the treatment of infectious diseases is the story of a treatment concept which dates back more than 120 years, to the late 19th century, and which originally, by the use of serum from immunized animals, provided the

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first effective treatment options against severe bacterial infections (1, 2). By immunizing horses with bacterial toxins from *Clostridium tetani* and *Corynebacterium diphtheriae*, Emil A. Von Behring and Shibasaburo Kitasato (3) generated serum containing antibodies capable of neutralizing the effects of the toxins produced by these bacteria and successfully provided treatment for these serious diseases where the pathogenesis is driven by the effects of the bacterial toxins. For his work on providing treatment for diphtheria, Behring received the Nobel Prize in Physiology or Medicine in 1901. These radical treatment results quickly prompted development of multiple additional serum therapies for the treatment of infectious diseases caused by, e.g., *Neisseria meningitidis, Haemophilus influenza*, and group A *Streptococcus*. Since serum therapy involved administration of large amounts of crude mixtures of animal proteins including antibodies, they were associated with side effects in the form of hypersensitivity and serum sickness (2).

Due to the crude and unpurified nature of these products, side effects were seen even when administering human serum preparations. Side effects were observed in up to 50% of patients and were considered to be caused by immune complex formations that resulted in symptoms such as rash, itching, joint pain, fever, and in serious cases hypotension and shock. However, due to the lack of alternative options, these treatments were, despite their side effects, widely used. Serum was normally administered by intravenous infusion in patients after a test for hypersensitivity where a small amount of serum was injected subcutaniously (1). As described above, serum therapy applied in these early days (late 19th century and early 20th century) involved preparations of serum from rabbits and horses immunized with the infectious agent or in live and/or neutralized versions or toxins from these (1). The costs of keeping the immunized animals and the production and potency testing of the materials made this a relatively expensive treatment. In 1891, data from Klemperer (4) showed serum therapy to protect rabbits from *Streptococcus pneumoniae* infection and paved the way for this type of treatment and for development of similar serum-based treatments of streptococcal infections in humans. When treating humans, early administration of serum could reduce mortality significantly down to around 5% compared to when administered 4 to 5 days after onset of symptoms, when serum treatment was largely without effect. This strongly indicated the need for quick diagnosis and quick treatment to control the infection before it got out of control. Consequently, in the absence of a specific diagnosis, mixtures of serum from immunizations with different serotypes were used to circumvent this need for early treatment without having a serotype-specific diagnosis. The understanding that different serotypes existed for pneumococci and that efficient treatment relied on using serotype-specific serum was being built up during the 1920s and 1930s through experience from extensive clinical trials.

By the end of the 1930s serum therapy was the standard of care for treatment of pneumococcal pneumonia. At that time, the efficacy and potency of the derived sera were assessed in mice, in "the mouse protection test" by testing survival after a concomitant intraperitoneal injection of a lethal dose of pneumococci and the serum to be tested. Due to the inherent variation in this test, efficacy and survival in two thirds of the animals was the acceptance criteria, and 10 times the lowest dose providing this was used for defining a unit of the serum. This allowed for large batch-to-batch variation, and the use of different strains of bacteria for immunization probably explains part of the missing responses observed (1). In the early 20th century a pandemic of meningitis in Europe and the United States, with mortality rates up to 80%, spurred the development of serum therapy treatment options. Although in the 1930s this became the recommended treatment in children assumed to be suffering from meningitis, failure to reduce mortality in several meningitis epidemics during that time raised doubts about the general applicability of serum treatment. In those days serum therapy often involved quite extensive procedures and infusion of large volumes of serum. The following example clearly illustrates this. Data from Flexner and Jobling (5) from treating meningitis in monkeys resulted in the development of sera from immunized horses for treatment in humans. The treatment protocol included lumbar puncture and withdrawal of more than 30 ml of spinal fluid representing an amount slightly larger than the expected amount of horse serum to be injected subsequently. This treatment involved such daily slow infusions of up to 30 ml of serum until the patient's condition improved. This treatment was used in outbreaks in New York in 1905 and 1906 and did markedly decrease mortality.

After the discovery of penicillin by Fleming in 1928, and the subsequent introduction of antibiotics in the 1930s, serum therapy was largely abandoned over a period of 10 years due to the availability of these new, more broadly effective and cheaper treatment options, which also had fewer side effects. Although improvements in the purification of antibodies had resulted in preparations with better safety and side effect profiles, high manufacturing costs and narrow specificity resulted

in antibody therapy being mostly restricted to a smaller number of selected treatments for snake venoms, bacterial toxins, and some viral infections (1, 2). Currently, antibody administration is used for treatment and prevention of hepatitis B virus, rabies virus, respiratory syncytial virus (RSV), *Clostridium tetani, Clostridium botulinum*, vaccinia virus, echovirus, and enterovirus. For the most part, these treatments consist of pooled immunoglobulin, also known as IVIG (intravenous immunoglobulin), from several postexposure donors. This results in both batch-to-batch variation, in the need for relatively large amounts of serum due to low specificity and to restricted supplies due to reliance on exposed donors.

However, several challenges have resulted in the need for new tools in the treatment and prevention of infectious diseases. The broad and general use of antibiotics in human and veterinary medicine for many years has resulted in the development of multi-resistant strains of bacteria with limited to no response to existing treatments such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *S. aureus*, and others. This has resulted in patients needing screening and treatment with several antibacterial agents and longer treatment time, causing extra strain on patients and health care providers (6; http://www.cdc.gov/drugresistance/threat-report-2013/index.html). According to the WHO and CDC more than 25,000 people in European Union countries and similar numbers in the United States die every year as a result of antibiotic-resistant infections. This together with the emergence of new pathogens (e.g., severe acute respiratory syndrome, Middle East respiratory syndrome), the re-emergence/epidemics of old/known pathogens (e.g., Ebola), and the difficulties in treating infections in immune-deficient patients (e.g., HIV patients) has highlighted the need for new solutions. The 2014 Ebola epidemic in West Africa (Liberia, Sierra Leone, Guinea, Nigeria, and Senegal) has further highlighted this. No treatment or prophylactic vaccine is available to treat or prevent the spread of Ebola infections, which have an average mortality of >50%. Local health authorities in the affected countries are struggling to contain and handle the disease, which is threatening to go out of control and spread more widely. Various products, mainly antibody cocktails from previously recovered patients, are being used despite a lack of clinical data on their safety and efficacy, and those are the only sporadically available treatment options and only in small amounts and for a few patients.

Ebola is an example of a disease which normally affects only a small number of individuals and which normally burns out when disease outbreaks are contained. Therefore, given the small number of potential patients affected by previous Ebola infections to date, there was no incentive for big pharma companies to do research and development of drugs for Ebola. With the increasing number of infected (13,567) and a death toll of 4,951 (7) and the lack of the ability to contain the epidemic, it will be interesting to follow the aftermath of this outbreak and see whether there will be requests for new ways to ensure that vaccines and treatment options are available for Ebola and similar high-mortality and potential bio-warfare infections that have no available treatments or procedures to for mass-production upon the first signs/reports of active infections. Although both the CDC and the WHO have special programs focusing on these types of infections, the Ebola outbreak in 2014 clearly shows that more financial support for research and development of new diagnostics and treatments is needed. This is one example where antibody-based treatments would have the potential to play a major role. To put this in the right perspective, one should, however, not forget that other infectious diseases such as tuberculosis, influenza, and malaria kill hundreds of thousands each year. There is therefore plenty of room for improvement in developing treatments for these diseases as well, but the high attention drawn to, e.g., the Ebola outbreak creates a special niche and opportunity where antibody treatments could gain extraordinary development funding and support and prove their value and treatment potential.

That antibody-based therapies could take this role is supported by the revolution in technologies for the development, selection, generation, and purification of fully human antibodies described in more detail later in the section "Methods and Platforms for Generating Antibodies". Antibody-based programs currently at different stages of development include investigations into the potential use of single antibody preparations, combinations of antibodies (to avoid survival of escape mutants), fragments of antibodies, and antibodies carrying radioactive isotopes or cytotoxic drugs or antibody-like frameworks (e.g., fibronectin) either alone, as a first-line treatment, or as an adjunct to existing treatments. This multitude of possible formats, the ability to raise antibodies to almost any target and the ability to engineer both size, effector functions, and half-life are now considered by many to provide very valuable tools for designing specific antibody-based treatments to eradicate specific targeted infectious agents. However, although several antibody-based therapies have been approved for oncology and anti-inflammatory

TABLE 1 Approved and pending antibody-based therapies

Name: antibody	Target: antibody type	Indication	Company	Approval date
OKT3[a]: Muronomab-CD3	CD3: murine, IgG2a	Autoimmune	Johnson & Johnson	1986 (U.S.)
ReoPro: abciximab	PIIb/IIIa: chimeric, IgG1, Fab	Homeostasis	Johnson & Johnson	1984 (U.S.)
Rituxan: rituximab	CD20: chimeric, IgG1	Cancer	Genentech	1997 (U.S.) 1998 (E.U.)
Zenapax[a]: daclizumab	CD25: humanized, IgG1	Autoimmune	Roche	1997 (U.S.) 1999 (E.U.)
Simulect: basiliximab	CD25: chimeric, IgG1	Autoimmune	Novartis	1998 (U.S., E.U.)
Synagis: palivizumab	RSV: humanized, IgG1	Infections	MedImmune	1998 (U.S.) 1999 (E.U.)
Remicade: infliximab	TNFα: chimeric, IgG1	Autoimmune	Johnson & Johnson	1998 (U.S.) 1999 (E.U.)
Herceptin: trastuzumab	HER2: humanized, IgG1	Cancer	Genentech/Roche	1998 (U.S.) 2000 (E.U.)
Mylotarg[a]: gemtuzumab ozogamicin	CD33: humanized, IgG4, immunotoxin	Cancer	Wyeth/Pfizer	2000 (U.S.)
Campath: alemtuzumab	CD52: humanized, IgG1	Cancer	Genzyme	2001 (U.S.) 2001 (E.U.)
Zevalin: ibritumomab tiuxetan	CD20: murine, IgG1, radiolabeled (yttrium 90)	Cancer	Biogen Idec	2002 (U.S.) 2004 (E.U.)
Humira: adalimumab	TNFα: human, IgG1	Autoimmune	Abbott	2002 (U.S.) 2003 (E.U.)
Xolair: omalizumab	IgE: humanized, IgG1	Autoimmune	Genentech/Roche	2003 (U.S.)
Bexxar: tositumomab-I-131	CD20: murine, IgG2a, radiolabeled (iodine 131)	Cancer	Corixa/GSK	2003 (U.S.)
Raptiva[a]: falizumab	CD11a: humanized, IgG1	Autoimmune	Genentech/Roche	2003 (U.S.) 2004 (E.U.)
Erbitux: cetuximab	EGFR: chimeric, IgG1	Cancer	Imclone/Lilly	2004 (U.S.) 2004 (E.U.)
Avastin: bevacizumab	VEGF: humanized, IgG1	Cancer	Genentech/Roche	2004 (U.S.) 2005 (E.U.)
Tysabri: natalizumab	α4-Intergrin: humanized, IgG4	Autoimmune	Biogen Idec	2004 (U.S.)
Actemra: tocilizumab	Anti-IL-6R: humanized, IgG1	Autoimmune	Chugai/Roche	2005 (Japan) 2010 (U.S.)
Vectibix: panitumumab	EGFR: human, IgG2	Cancer	Amgen	2006 (U.S.)
Lucentis: ranibizumab	VEGF: humanized IgG1 Fab	Macular degeneration	Genentech/Roche	2006 (U.S.)
Soliris: eculizumab	C5: humanized IgG2/4	Blood disorders	Alexion	2007 (U.S.)
Cimzia: certolizumab pegol	TNFα: humanized, pegylated Fab	Autoimmune	UCB	2008 (US)
Simponi: golimumab	TNFα: human IgG1	Autoimmune	Johnson & Johnson	2009 (U.S., E.U., Canada)
Ilaris: canakinumab	IL1b: human IgG1	Infalmmatory	Novartis	2009 (U.S., E.U.)
Stelara: ustekinumab	IL-12/23: human IgG1	Autoimmune	Johnson & Johnson	2008 (E.U.) 2009 (U.S.)
Arzerra: ofatumumab	CD20: human IgG1	Cancer	Genmab	2009 (E.U.)
Prolia: denosumab	RANK ligand: human IgG2	Bone loss	Amgen	2010 (U.S.)
Numax: motavizumab	RSV: humanized IgG1	Anti-infective	MedImmune	Pending
ABthrax: Raxibacumab	*B. anthrasis* PA: human IgG1	Anti-infection	GSK	2012 (U.S.)
Benlysta: belimumab	BLyS: human IgG1	Autoimmune	HGS	2011 (U.S.)
Yervoy: ipilimumab	CTLA-4: human IgG1	Cancer	BMS	2011 (U.S.)
Adcetris: brentuximab vedotin	CD30: chimeric, IgG1, drug- conjugate	Cancer	Seattle Genetics	2011 (U.S.)
Perjeta: pertuzumab	Her2: humanized, IgG1	Cancer	Genentech/Roche	2012 (U.S.)
Kadcyla: ado-trastuzumab emtansine	Her2: humanized, IgG1, Drug-conjugate	Cancer	Genentech/Roche	2013 (U.S.)
Raxibacumab	Anti-B anthrasis PA: human IgG1	Anthrax infection	Not approved	2012
Entyvio: vedolizumab	Integrin $\alpha_4\beta_7$: humanized IgG1	Crohn's disease, ulcerative colitis	Takeda	2014 (U.S.)

(continued)

TABLE 1 Approved and pending antibody-based therapies *(continued)*

Name: antibody	Target: antibody type	Indication	Company	Approval date
Cyramza: ramuciruumab	Anti-VEGFR2: Human IgG1	Gastric cancer	Lilly	2014 (U.S.)
Gazyva: obinutuzumab	Anti-CD20: humanized IgG1 glucoengineered	Cancer/CLL[b]	Genentech/Roche	2014 (U.S.)
Sylvant: situximab	Anti IL-6: chimeric IgG1	Castleman disease	Janssen	2014 (U.S.), 2014 (E.U.)
Cosentyx: sekukinumab	Anti IL-17a: human IgG1	Psoriasis	Novartis	2015 (U.S.), 2015 (E.U.)
Nivolumab	Anti PD-1: human IgG4	Melanoma	BMS	Not approved
Keytruda: pembrolizuumab	Anti-PD-1: humanized IgG4	Melanoma	Merck	2014 (U.S.)

[a]Antibodies approved but later withdrawn.
[b]CLL, chronic lymphocytic leukemia.

indications (see Table 1), only one monoclonal antibody (mAb) is approved against an infectious disease agent—Synagis (palivizumab, MedImmune) for the prevention and treatment of respiratory syncytical virus (RSV) in high-risk children. Economic obstacles such as the high cost of mAb therapies and relatively small markets have resulted in less interest from pharmaceutical companies in developing these. Also highlighting the challenges in developing these types of antibody-based treatments are the difficulties encountered in developing a follow-up higher-affinity candidate to Synagis. Poor translatability of data obtained in the available animal disease models for RSV in cotton rats has resulted in nonapproval due to inferiority relative to Synagis. In other viral diseases such as cytomegalovirus (CMV) the high species specificity of the strains of CMV makes it impossible to test human antibody therapies *in vivo*. Therefore, any real data on the efficacy of treatments for CMV will not be obtained until testing in clinical trials. As described later in this article several new antibody-based therapies for infectious diseases are in development, and hopefully this will gradually open the field and result in better treatment options and outcomes for patients.

INTRODUCTION TO ANTIBODIES

Antibodies, also called immunoglobulins based on their combined structure and function in immune responses, are produced by B cells of the immune system. They are part of the adaptive immune response and are specially designed for neutralizing and eliminating the infectious agents and toxins produced by these. Antibodies are present in blood, plasma, and extracellular fluids, and since these fluids were formerly known as humors, they were said to be part of the humoral immune response. Antibodies are Y-shaped structures consisting of two main parts: the upper arms of the Y, which contain two identical variable-region antigen binding sites, and the lower region, called the constant region, which is responsible for the initiation of effector functions that lead to the removal and destruction of the pathogen or cells harboring the pathogen (see Fig. 1). The antigen-binding sites on an antibody can by themselves bind to and neutralize bacterial toxins and viruses, thereby preventing them from binding to their target cells or receptors causing toxic effects or spread of the infection.

Antibodies consist of two pairs of heavy and light chains which, as described above, are held together in a Y-shaped arrangement. Each of the heavy and light chains in these pairs is separated into constant and variable chains. The upper arms of the Y each contain a variable and a constant section of the light and the heavy chain, where the upper variable parts of the heavy and light chains contain the antigen-binding site, and the constant parts are connected via disulfide bonds. The lower part of the Y, called the constant part, consists of two or three constant segments (immunoglobulin domains) from each of the two heavy chains interacting and also linked via disulfide bonds. The sections of the heavy chains connecting the constant part of the upper arms to the constant parts of the constant region contain special hinge regions that provide flexibility to the different sections of the antibody to bind to antigens and effector cells. The constant regions of the heavy chain also contain attached oligosaccharide moieties which provide functional specialization to the antibodies (8).

The variable domains of the heavy and light chains form the antigen-binding sites and contain special hypervariable segments called complementarity-determining regions (CDRs), which allow the B cells, via genetic recombination, to generate antibodies to all the different antigens specific to amino acid sequences or three-dimensional structural motifs (polysaccharides, DNA, and RNA) found on pathogens.

Based on the structure of their heavy chain constant regions, antibodies, or immunoglobulins, are separated into five classes—IgM, IgG, IgE, IgD, and IgA. Differences in the sequences of the constant regions provide

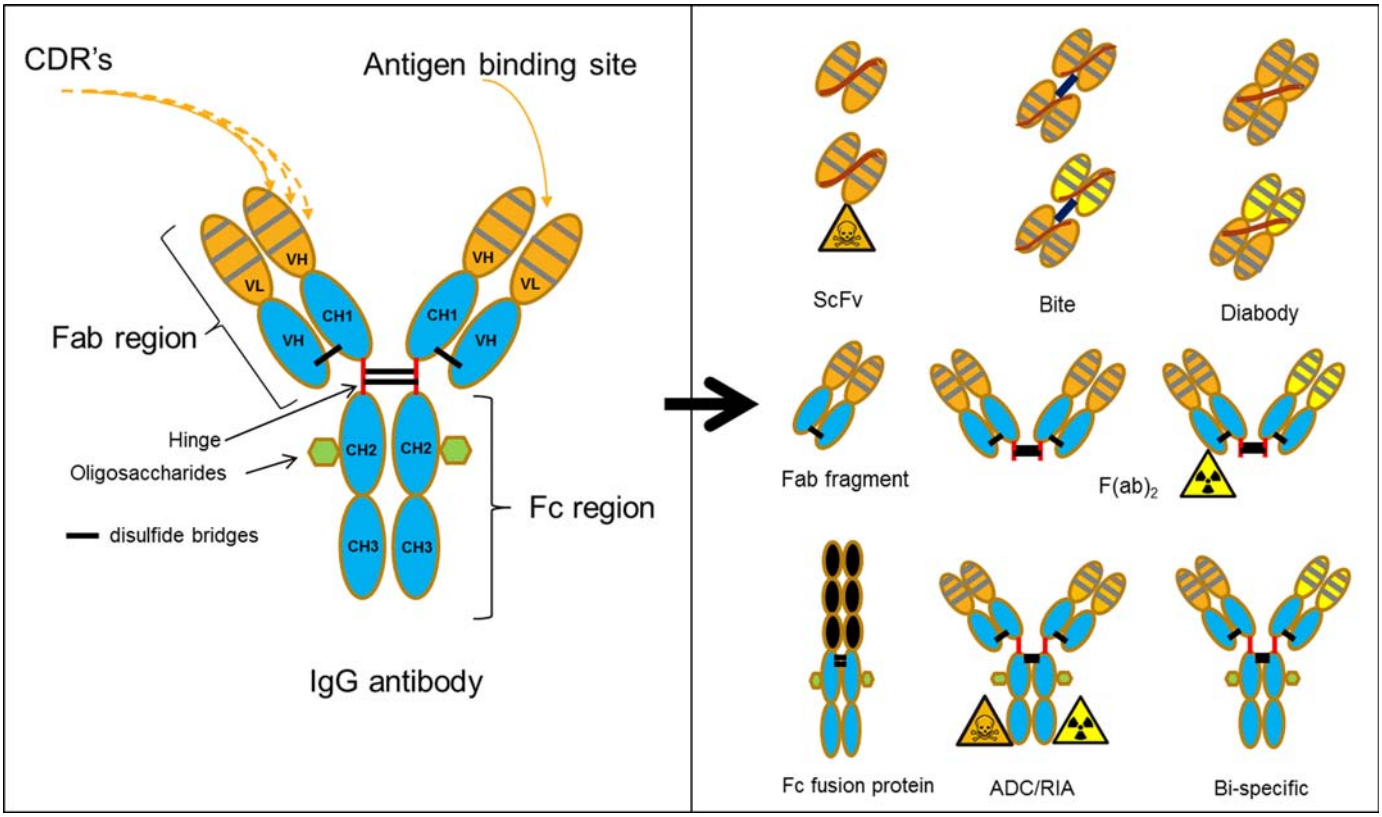


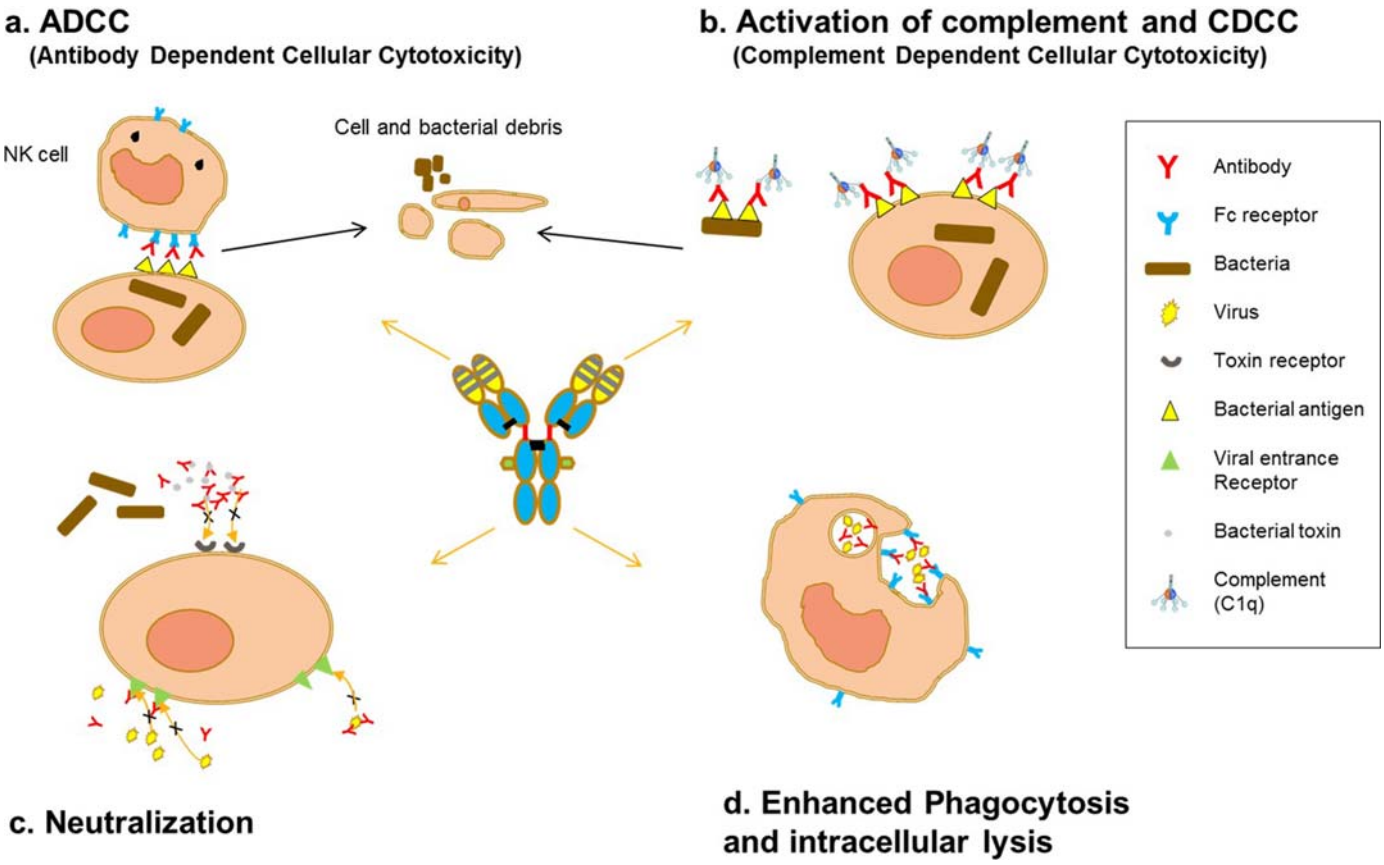
FIGURE 1 (Left panel) Model of antibody structure exemplified by IgG. On top the antigen-binding sites in orange each contain one variable light and variable heavy domain with the three complementarity determining regions (CDRs) that are responsible for the specific binding of the antibody to its target. For each arm of the antibody, an additional set of variable heavy and light domains, together with the CDR-containing domains, represent the two fragment antigen binding (Fab) regions. The two Fabs are held together via two disulfide bridges. Below the Fabs is the Fc region, which contains four constant heavy domains. On the upper pair of these domains are binding sites for oligosaccharides, which have major importance for the ability of the antibody Fc part to trigger effector functions when the Fc portion is bound to Fc gamma receptors on natural killer cells, neutrophil granulocytes, monocytes/macrophages, dendritic cells, and B cells. (Right panel) Examples of some of the antibody-derived alternative formats used to exploit the specific features of the CDRs, the Fabs, and the Fc parts of the antibodies. ScFv: The single chain fragment variable consists of the variable domains of the heavy and light chains held together by a flexible linker. This can also be used as a carrier of a cytotoxic drug in a so-called antibody drug complex (ADC) where the specificity of the ScFv is used to target the cytotoxic drug to, e.g., a tumor. Bite (bi-specific T cell engager): Fusion proteins consisting of two ScFvs, one directed against the target on a tumor cell and the other against the T cell receptor (CD3). Diabody: ScFv dimers where short linker peptides (five amino acids) ensure dimerization, and not folding, of the ScFvs. Fab and $F(ab)_2$ fragments: Single Fab fragments or fragments containing two Fabs linked via disulfide bridges. This is used where effector functions related to the Fc part of the antibody are unwanted and where a smaller size is desired to obtain better tissue penetration in, e.g., tumors. Due to the lack of the FcRn binding via the Fc part, Fab and $F(ab)_2$ fragments have much shorter half-lives (hours or days) than full-size antibodies (weeks). These can also be used as carriers of cytotoxic payloads or cytotoxic radioactive isotopes and for the $F(ab)_2$ fragments can be constructed as bi-specifics which can cross-link immune cells and target cells. Fc fusion protein: Fusion protein containing the Fc domain of an immunoglobulin bound to a peptide. The peptide can be a ligand for a specific receptor on a target cell or a blocking peptide for a soluble ligand. The Fc part provides a longer half-life to the construct and the potential to bind to and engage effector functions in the killing of, e.g., tumor cells or infected cells. ADCs/RIAs and bi-specifics: Full-size IgG antibodies carrying either a cytotoxic chemical or radioactive payload, which may also carry different CDRs, enabling cross-linking of effector and target cells for increased killing. doi:10.1128/microbiolspec.AID-0026-2014.f1

distinct characteristics to these different classes. These characteristics include the number of heavy chain constant segments, the number and location of interconnecting disulfide bridges, the length of the hinge region, and the number and location of oligosaccharide moieties. For IgG, four heavy chain versions exist, creating IgG1, 2, 3, and 4 isotypes, each with different characteristics in their serum half-lives and ability to trigger different

effector functions. Because of the combined advantage from ability to trigger effector mechanisms, long [21 days] half-life, their ability to be transported over the placenta, and their stability in the production process, antibodies of the IgG class and of the IgG1 isotype are preferred as the basis for new antibody-based therapies. The effector functions are triggered by the Fc part of an antibody when the variable parts of the antibody are bound directly to an infectious agent or to proteins from an infectious agent expressed on an infected cell. This binding leaves the Fc part free to interact with Fc receptors on phagocytic cells and neutrophils, eosinophils, and natural killer cells capable of inducing phagocytosis or lysis of infectious agents or infected cells. These effector functions include (i) the ability to activate the complement system for lysis (complement-dependent cellular cytotoxicity); (ii) triggering of uptake and destruction by phagocytic cells such as macrophages and neutrophils via interaction with Fc receptors on these cells; (iii) activation of macrophages, eosinophils, neutrophils, and natural killer cells to kill infected cells via binding to Fc receptors on these cells and antibody-dependent cellular cytotoxicity; and (iv) neutralization of bacterial toxins and blocking of binding and uptake of bacteria or viruses to target cells (see Fig. 2) (9).

The Fc part of IgG antibodies also contains a region that interacts with the neonatal Fc receptor (FcRn) expressed on most immunocompetent cells and in many

FIGURE 2 Effector functions of antibodies. (a) Antibodies bind to pathogen-derived or endogenous antigens expressed on the surface of an infected cell, which triggers binding to Fc receptors on natural killer cells and lysis of the infected cell by antibody-dependent cellular cytotoxicity. (b) Antibodies bind to pathogen-derived or endogenous antigens expressed on the surface of infected cells, which triggers activation of complement through binding of complement factor C1q. (c) Neutralization. Top: Bacterial toxin neutralized by bound antigen. Bottom: Antibody bound to either receptor for the virus or to the virus itself, which blocks virus binding and entry into the cell. (d) Antibody bound to viral surface proteins binds to Fc receptors on phagocytic cells, e.g., macrophages, and triggers endocytosis and destruction of virus in endolysosome. doi:10.1128/microbiolspec.AID-0026-2014.f2



other cells such as endothelial cells in kidney, liver, placenta, lung, and breast (10), which protects antibodies from degradation by phagocytotic/exocytotic cycling into FcRn-expressing cells, thereby prolonging their half-life. This results in half-lives of 20 to 21 days for most IgG1, 2, and 4 antibodies, whereas half-lives are 10, 6, and 2 days, respectively, for IgM, IgA, and IgE. The highly specific binding sites on the variable regions of the upper arms of the antibody allow for several important features of antibodies–binding to and neutralization of, e.g., bacterial toxins, binding to pathogens and blocking their binding to or interaction with target cells (e.g., cyncytia formation of CMV-infected cells); or blocking of binding and interactions of infectious agents with target cells via blocking receptors on the target cells.

METHODS AND PLATFORMS FOR GENERATING ANTIBODIES

As described above, the early use of passive antibody transfer as a therapy in infectious diseases involved the use of immune serum from immunized sheep and horses. These preparations were crude and contained a broad mixture of neutralizing and non-neutralizing antibodies and both the foreign nature of the antibodies and the numerous host serum proteins. This resulted in less specific and thus less efficient therapies due to a high variability in efficacy between batches derived from different animals immunized with different strains of bacteria in different laboratories and resulted in multiple unwanted side effects related to hypersensitivity and anaphylactic reactions to foreign proteins. These days, antibodies being developed for treatment of diseases in humans are highly purified and are mostly fully human monoclonal antibodies. The term monoclonal antibody refers to that cell cultures used for generating these antibodies each originates from a single cell and thus produces only one specific antibody. Development of fully human monoclonal antibodies happened in stages over a time span of more than 15 years. First stage in this process was generation of monoclonal murine IgG antibodies (e.g., OKT3 [muronomab-CD3, Johnson and Johnson] approved in 1986). Next stage was generation of so called chimeric antibodies consisting of human heavy and light chain constant sections but with murine variable sections (e.g., Rituxan [rituximab, Genentech]). Next stage was then the humanized antibodies where the only remaining murine part of the antibody is the antigen-binding CDR (e.g., Xolair [omalizumab, Novartis/Genentech]). Both the first chimeric and humanized antibodies were approved in the late 1990s. Then finally 16 years later, the first fully human antibody Humira (adalimumab, Abbott) was approved in 2002. Since the time of the crude and low-scale manufacturing of the original antibody serum products, much has changed, and therapeutic antibodies are now produced by fermentation in 5,000 liter scale using highly controlled and documented yeast or mammalian cell lines that are genetically engineered to express a single specific antibody. This has resulted in a pure and highly specific antibody product, and thanks to great improvements in cell line design and fermentation efficiency, monoclonal antibody therapeutics can now be produced at more reasonable, although still high, costs.

The price of antibody-based therapies is, however, still much higher than chemically synthesized antibiotics. Although they do provide very welcome and needed treatment options, the cost of antibody-based treatments approved for cancer and inflammatory diseases is creating major economic challenges for health care systems and health care providers around the world. Targeted immune therapy in, e.g., colorectal cancer with 8 weeks of treatment with a monoclonal antibody like Erbitux (cetuximab, Imclone/Lilly) cost up to $31,790 in the 1990s. In comparison, $63 for an 8-week treatment with fluorouracil, which was the standard treatment until the mid-1990s, puts the cost of antibody treatments in perspective.

Highly specific antibodies monoclonal antibodies (each produced by culture of cells all derived from the same single cell), can be selected for and generated from immunized humans or animals in several different ways: (i) phage display of a human variable light segment library from several donors and B cells, followed by cycles of panning against target antigen, selection of phages with the desired target specificity, and subsequent cloning and expression in a cell line for expression; (ii) the use of transgenic mice carrying the genes for human IgG and immortalization of the mouse B cells by fusing them with myeloma cells (the hybridoma technology); and (iii) isolation of memory B cells or activated memory B cells (plasmablasts) from patients that have been or are exposed to the infectious agent. After isolation the memory B cells are then first screened for reaction to the relevant antigen and positive cells are then immortalized by transformation with Epstein Barr virus transformation in the presence of a oligodeoxynucleotide (CpG) and irradiated peripheral blood mononuclear cells (11, 12). Finally the cell cultures are subjected to several steps of "limiting dilution" where the concentration of cells in the subsequent seeding cultures are less than one. This is

done to ensure that each cell culture is derived from one cell only (monoclonal) and that only identical antibodies from a single clone is harvested from each of the cell cultures.

An essential technological step on the way to the current antibody products was the method to produce monoclonal antibodies by immortalizing B cells, which is the basis for the "hybridoma technology" (13). This allowed the production of large amounts of homogenous antibodies with defined specificity and a single Ig class and isotype (14). This was quickly adapted for clinical use, and in the 1980s the OKT3 anti-CD3 monoclonal murine IgG2 antibody was the first one in the class approved for prevention of organ transplant rejection (14). The ability to generate monoclonal antibodies in unlimited amounts and against almost any target protein provided extremely valuable tools for detecting, locating, inhibiting, and blocking specific markers and pathways in general biological and medical research and in setting up analyses for numerous markers via enzyme-linked immunosorbent assay or immunohistochemistry. It also played a major role in basic research into the mechanisms of antibody action.

STRATEGIC CONSIDERATIONS IN DEVELOPING ANTIBODY-BASED THERAPIES FOR INFECTIOUS DISEASES

As mentioned above, the past 10 to 15 years saw a revolution in development and approvals of monoclonal antibody-based therapies for inflammatory and neoplastic diseases. This revolution, however, did not include treatments for infectious diseases. As can be seen in Table 1, more than 40 antibody-based treatments have been approved or are under final evaluation. Although many products for infectious diseases are in different stages of development, still only one monoclonal antibody-based product, Synagis, is currently approved for use in infectious diseases. The main reason for this is the availability of the much cheaper, easier to administer, and more broadly acting antimicrobials or antibiotics. However, the increasing development of resistant strains of bacteria (e.g., MRSA) and viruses has opened the door for a re-emergence of antibody-based therapies. Several features of monoclonal antibodies are, however, working against them when comparing with antimicrobial therapies. First, and most importantly, is the very high cost of production already mentioned above. In addition, since antibodies are proteins, they need to be treated carefully, kept refrigerated, and they are administered by intravenous or subcutaneous injection. In contrast, antibiotics usually come in the form of tablets that can be taken orally and can be kept in a bag or in a closet at room temperature. Antibiotics normally target general mechanisms, e.g., cell wall formation in bacteria, and can act against, e.g., a broad spectrum of bacteria, whereas antibodies are very specific to a single virus, bacteria, or bacterial subtype, and a clear diagnosis should be made before initiating treatment with a monoclonal antibody. This highlights the need for improving or developing diagnostics in parallel with developing antibody therapies. The specificity also has an economical angle since it results in a smaller market compared to broad-spectrum antibiotics, making antibody-based therapies less attractive for pharmaceutical companies to develop.

The specificity is, however, also a strength in the sense that the antibody works only on a specific infectious agent, and although mutations in that agent can render the antibody ineffective, this does not affect other similar agents and thus does not cause the spread of resistance. The specificity also results in very low off-target binding and therefore very few side effects, including the gastrointestinal effects often observed with antibiotics due to broad effects on bacterial flora in the gut. The balance between antibody therapies and antibiotics with respect to costs and ease of use in the clinic should, however, be seen in a broad and more realistic context, including the current situation around the development of resistance, which has resulted in antibiotic therapy in the United Kingdom not working in one in seven patients (15). In the United States this situation has resulted in multiple cases with the need for dosing up to 10 different antibiotics before proper control of infection can be obtained. Table 2 lists the pros and cons of antibody-based therapies and antibiotics.

To make antibody therapeutics more efficacious and more convenient for patients and treating physicians, several approaches have been and are being taken.

Strategy To Avoid Escape Mutants

Although antibody treatments do not induce resistance in nontarget bacteria or viruses, both bacteria and viruses have the ability to escape the host immune system and specific antibody treatments via mutations that change their surface proteins or structure, creating so-called escape mutants that are no longer neutralized by the specific antibody. The best way to circumvent this is by using a combination of antibodies directed at different viral targets. The use of cocktails of two or more antibodies was shown to provide synergism or additive effects in neutralizing, e.g., hepatitis B virus (HBV) and RSV infections (16). Combinations of antibodies have also been

TABLE 2 Pros and cons of antibody based therapies related to serum therapy and antibiotics

Parameter	Serum therapy	Antibiotics	mAbs	Comments
Source	Animals Humans	Chemical synthesis Fermentation	Tissue culture Bioreactor Fermentation	
Lot variation	High	Low	Low	
Specificity	Narrow	Broad	Narrow	Narrow specificity prevents development of resistance in non-target species but results in the requirement for a specific diagnosis before treatment. Restricts treatment to single species.
Toxicity	High	Low	Low	Original crude antibodies from animals had poor tolerability, but current human- and animal-derived immunoglobulins have been proven safe
Ease of administration	Difficult (intravenous [i.v.], intramuscular [i.m.], subcutaneous [s.c.])	Easy/difficult (oral/i.v.)	Easy (i.v., i.m., s.c.)	Many antibiotics can be administered orally, whereas serum or mAbs are given by i.v or s.c injection
Pharmacokinetics	Variable	Consistent	Consistent	
Cost/dose	High (in the hundreds)	Very high (in the thousands)	Low (in the single digits)	

used in HIV, targeting GP41 and GP120 viral proteins (17), and rabies (18) and are part of the strategy in most pharmaceutical companies that are developing antiviral antibodies. Therefore, part of the early preclinical development of such antibody combinations consists of serial passage of virally infected cells (>20 generations) to ensure continued efficacy and the absence of escape mutants. This is combined with testing against known patient isolates, when available. Cocktails of antibodies would also be an interesting approach to target groups of infectious agents often seen in parallel in, e.g., burn wounds.

Antibody Engineering

Another strategy to increase efficacy, increase the ability of the treatment to reach the intended target, and avoid unwanted side effects resulting from the killing of non-target cells is antibody engineering—more precisely, genetic manipulation of the Fc domain (mainly in the CH2 domain) or changes to the glucosylation pattern of the N-linked oligosaccharide moieties attached at antibody N297 in the Fc part of the heavy chain. For generating antibodies with enhanced effector functions, different mutations have been identified that have increased affinity to the FcγIIIa receptor and a significant enhanced cellular cytotoxicity (S239D/A330L/I332E, also known as 3M [19, 20], F243L [21], and G236A). These antibodies either directly or indirectly enhance binding of Fc receptors and thus significantly enhance cellular cytotoxicity. Enhanced effector function can also be achieved by modulating the oligosaccharide moieties. Removal of fucose from the A297 linked oligosaccharide moietites, which creates so-called afucosylated Fc domains, has been shown to greatly increase the potency for inducing antibody-dependent cellular cytotoxicity (22). This is achieved by manufacturing the antibodies in cell lines lacking the enzyme fucosyl transferase, which renders them unable to add fucose to the oligosaccharide moieties (22).

Similarly, ways to reduce or ablate the ability of antibodies to trigger effector functions have been described and are being used broadly in cases where the aim is to block specific membrane-bound receptors/targets and where killing of the cell harboring the target is not desired. Again, mutations in the Fc part, e.g., the mutations L234A and L235A, also called the LALA mutation, greatly reduce but do not completely remove effector functions by removing amino acids important for the C1q factor of complement (23, 24). Modulation of the glucosylation pattern, in this case creating completely aglucosylated antibodies, has also been shown to remove the ability to properly bind Fc receptors on effector cells and trigger effector functions. One alternative approach used especially when developing immunomodulatory agonistic antibodies is the use of antibodies of the IgG4 isotype, which does not trigger effector functions. Finally, mutations in the Fc part that increase the affinity to the FcRn receptor have also been used to create antibodies with an increased half-life. Introduction of three mutations in the Fc domain (M252Y,

S254T, and T2556E, also called the YTE) has been shown to provide a half-life extension of 3- to 4-fold (25). From a convenience point of view, a long half-life is obviously attractive, but it can be a down-side in the case of severe adverse effects due to the long duration of action.

Creating "Magic Bullets"

In oncology, to enhance the efficacy of therapeutic antibodies, the tactic of using the specific binding capacity of the antibody to deliver a cargo in the form of a cell toxin or a radioactive isotope to kill tumor cells has long been recognized. The first treatments using radiolabeled antibodies were the anti-CD20 antibodies Zevalin (ibritumomab tioextan; yttrium 90 labeled) and Bexxar (tositumumab; iodine 131 labeled), which were approved in the United States in 2002 and 2003, respectively. These demonstrated the potential for developing such antibodies. However, they are not widely used in cancer therapy. Successful generation of labeled antibodies against fungal infections with *Cryptococcus neoformans* and *Histoplasma capsulatum* and bacterial infections with *S. pneumoniae* was reported by Saylor et al. (26), and efficacy was shown *in vitro* and in animal models. However, clinical efficacy still needs to be demonstrated. One opportunity with this approach is that these antibodies can be directed at infected cells and not the pathogen itself, killing the infected cells and thereby potentially removing reservoirs of infected cells (26). The challenges in selecting the right isotopes, achieving distribution and uptake into tumors and tissues, and minimizing exposure of non-target tissues have slowed the pace of development of these types of antibody treatments. Difficulties in handling and disposing of such products have also contributed to this.

With a similar approach but with antibodies aimed at delivering cytotoxic drugs via antibodies also known as antibody-drug complexes (ADCs) there is currently great enthusiasm and activity, especially in oncology. The first antibody of this kind, Myoltarg (gemtuzumab ozogamicin), was approved by the FDA in 2000 but was later withdrawn due to major toxicities in patients, caused by instability and heterogenicity. Two other ADC antibodies have been approved for the treatment of cancer: Adcetris (brentuximab vedotin) and Kadcyla (ado-trastuzumab emtansine), which were approved in 2011 and 2013, respectively. The ADC field is expanding, and many products are in development in cancer and inflammatory diseases, but has not resulted in any approvals for products in infectious diseases. A major challenge in this approach is the selection of the right linker to create a stable complex, as well as ensuring minimal off-target exposure and, most importantly, achieving internalization and release in the proper compartment of the target cells. The antibody CDRs should ensure specific binding and delivery of the cytotoxic drugs, but in animal studies using a specific ADC and an ADC without target but labeled with same toxin, the same broad pattern of toxicity was observed with both products. This indicates that the desired specific targeting to mainly affect target cells or tissues is currently not achievable. So far, this concept is used only in cancer treatment; for any successful development of such treatments in infectious diseases, a critical case-by-case risk/benefit evaluation combined with considerations of availability of other effective drugs will be needed.

Antibody Formats

In the era of antibody-based therapies, full-size antibodies were and are still the main format. There is, however, an increase in companies offering/researching into new variants of antibodies or new combinations of antibody-derived structures. Examples of these can be seen in Fig. 1 and include both bispecific full-size antibodies with antigen-binding sites with different targets, Fab or $F(ab)_2$ fragments, single-chain variable fragments (ScFv), pairs of ScFvs linked in different ways (diabodies and bites), fusion-proteins containing, e.g., a receptor fused to an antibody Fc part, and many others not shown. Much creativity has been demonstrated in this field of research, and every imaginable variation and combination of antibody structures is being created and tested. This is done to test how these different formats and the attributes of the different parts of antibodies potentially can overcome the challenges facing the development of antibody-based therapies. These challenges include the high costs of production and finding optimal combinations of size, stability, half-life, efficacy, and very importantly, safety. These new formats also introduces new structures foreign to the immune system and thus immunogenicity is likely going to be a major factor in these developments with the potential risk of loss of efficacy, loss of exposure, potential hypersensitivity reactions, and potential cross-reactivity to endogenous receptors or immunoglobulins. As described above, a lot of work was put into reducing immunogenicity and immune reactions to the animal-derived or chimeric antibodies by turning them into more and more fully human antibodies. It is therefore a paradox that with the introduction of these new formats (e.g., new scaffolds with fibrinogen carrying

antigen-binding sites), the risk of immunogenicity and immune reactions is being reintroduced. Time will show whether the safety and efficacy of these new formats will actually support their registration and use in patients with infectious diseases.

EXAMPLES OF ANTIBODIES THAT ARE OR WERE IN DEVELOPMENT FOR INFECTIOUS DISEASES

When searching for antibody-based treatments in different stages of development, it becomes obvious that the vast majority of these focus on viral and some on bacteriological infections. Around 70% of these focus on five main pathogens: hepatitis C virus (HCV), HIV, *Bacillus anthracis*, *Escherichia coli*, and *S. aureus*. The following section describes examples of projects targeting infections with these and a few additional treatments divided into antibacterials, antivirals, and antifungals.

Antibacterials

Due to their ability to cause a multitude of serious infections and due to their high propensity for developing resistance, MRSA infections are a major problem in hospitals and in local community settings. However, despite several clinical trials with antibodies targeting the clumping factor A, e.g., Aurexis (tefibazumab, Inhibitex) (27) and Aurograb (Novartis), a single-chain variable fragment (ScFv) targeting an *S. aureus* surface protease has failed to show efficacy of any of these treatments (28). Some hope has arisen from the work on pagibaximab (Biosynexus), which is an antibody for the prevention of staphylococcal sepsis in premature infants with low birth weight. Clinical phase I and II trials have shown good safety and no cases of treatment related adverse events when doses of 90 mg/kg were given. A phase III study is ongoing (29).

Research into mAbs targeting the adhesins SA I and II from *Streptococcocus mutans*, which aims at recolonizing the bacterial flora in the oral cavity to prevent caries, did not show the expected effects in the first human trials, and further development work was stopped (30).

Some optimism has been generated in finding an antibody-based treatment of *Pseudomonas aeruginosa*. Two antibody-based treatments are in development for the prevention and treatment of infections with *Pseudomonas* in ventilated patients: a full-size antibody, panobacumab (Kenta Biotech), directed against LPS 011, and a pegylated Fab product targeting the type 3 secretion system. Both of these have shown good tolerability and efficacy in patients, and phase II studies are ongoing (31, 32).

B. anthracis causes highly lethal pulmonary infections and is recognized as a potential biological warfare weapon. Four antibodies, raxibacumab (ABthrax, Human Genome Sciences), Valortim (PharmAthene/Medarex), Thravixa (Emergent Biosolutions), and Anthim (Elusys Therapeutics), are human antibodies directed against the protective antigen which interacts with the toxin's lethal factor and edema factor to facilitate their toxic action. All are undergoing clinical trials for tolerability, but raxibacumab was approved in 2012 for treatment of inhalation anthrax. It was, however, not a traditional approval based on phase II and III clinical trials showing efficacy but was the first treatment approved under the so-called animal rule, where the efficacy, for good reasons, is approved based on animal studies.

For treatment of infections with *Clostridium difficile* a cocktail of two antibodies, CDA-1 and CDB-1 MK3415A (Merck), for neutralization of toxins A and B are in phase II development (33). Clinical trial data with MK3415A given on top of a standard of care showed a lower recurrence rate compared to the standard of care alone. This was an example of positive data where a therapeutic antibody treatment was given as an adjunct to standard of care antibiotics.

Another cocktail of two antibodies (caStx1, caStx2; Shigamabs [LFB Biotechnologies/Thallion Pharmaceutics]) is being developed to treat infections with strains of *E. coli* producing Shiga toxin. There is currently no treatment for the hemolytic uremic syndrome caused by infections with these strains of *E. coli* which affects around 300,000 people every year. Phase I trials showed the cocktail to be well tolerated in healthy volunteers, and a phase II study is looking at pharmacokinetics and efficacy of treatment (34).

Antivirals

Synagis (palivizumab, MedImmune), for the prevention of RSV infection in infants, is currently the only approved monoclonal antibody treatment for infectious diseases. An affinity maturation and half-life extended third-generation monoclonal, Numax (motavizumab, AstraZeneca/MedImmune), is currently in clinical development but due to safety issues and an inability to demonstrate superiority has so far not been approved by the FDA. This is an interesting example of a case where a back-up for antibody, despite higher affinity and potency, fails to show improved efficacy in clinical trials (35).

A number of antibodies against HCV are in development. One of these (MBL-HCV-1) is directed against an envelope protein on the virus (36). The others are directed against host cell proteins, and one of these, bavituximab (Peregrine Pharmaceuticals) (37), is directed against phosphatidylserine, which normally is expressed only on the inner surface of the cell membrane but which in cancer- and virus-infected cells flips out and gets expressed on the external part of the membrane, allowing its use as a target for mAbs. Another approach adopted from cancer treatment is immunomodulation. Another example of a mAb being tested in HCV is the two anti-PD-1 receptor antibodies, CTZ-11 and BMS-936558, where blocking the inhibitory receptor PD-1 on CD8 T cells restores the ability of these cells to lyse HCV-infected cells.

Shifting the focus to HIV, a number of mAbs which targeted the GP120 envelope protein were being tested but suffered from escape mutants causing a rebound of viral loads. A different strategy targeting conserved receptors such as CCR5 or CXCR4, which serve co-receptors for the virus on target CD4 T cells, has had some success in reducing viral loads (38, 39). Two such antibody therapies, Ibalizumab (WuXi Pharma Tech) and PRO140 (CytoDyn), are in late-stage clinical development and have caused some modest optimism.

In rabies the current treatment practice after potential exposure is vaccination and administration of immunoglobulin from vaccinated humans (human rabies immunoglobulin [HRIG]) or from horses. In Asia >50,000 people annually die from rabies (35), and a shortage of specific immunoglobulins has resulted in the testing of a polyclonal antibody product (CL-184, foravirumab, Sanofi/Crucell); virus-neutralizing effects similar to what is observed with immunoglobulin treatment in dosed humans has been observed (40).

Antifungal

Fungal infections with yeasts such as *Candida albicans* and *C. neoformans* cause morbidity and mortality in immunocompromised individuals. For candidiasis one antibody, efungumab (Mycograb, Novartis), which targets heat-shock protein 90 (HSP90), was in late-stage clinical development for treatment on top of antifungal therapy. However, safety concerns and lack of proven efficacy compared to antifungal treatment alone resulted in discontinuation of the program. For *Cryptococcus* a murine antibody, MAb18b7, which targets capsular glucuronoxylomannan and acts through complement activation, was tested in HIV patients, but due to generation of antimouse antibodies, this program was halted (41).

FUTURE PERSPECTIVES FOR ANTIBODY TREATMENTS IN INFECTIOUS DISEASES

There is no questioning the fact that new treatment options are needed in infectious diseases. Increasing multidrug resistance, inability to treat immunocompromised patients, risk of bioterrorism, and new emerging diseases call for alternatives to the current armament. For that purpose, antibodies and antibody-derived treatments offer very attractive tools and attributes to kill or neutralize infectious agents, lyse infected cells, or modulate the immune system to enable effector cells to escape immunosuppressed conditions and contribute to the elimination of infections. The ability to raise antibodies to any target, and the ability to modulate effector functions, half-life, and size of the treatment units makes antibodies ideal for tailoring treatments for specific infectious agents.

Antibody-based treatments have been going through a time of great excitement and development in generating treatments for cancer and inflammatory diseases, and many new antibody-based products are now approved for clinical use (Table 1). This development passed by the field of infectious diseases, and despite multiple programs in multiple diseases only one treatment, the anti-RSV antibody Synagis, has been approved. Inherent challenges such as high cost, the need for parallel development of specific diagnostics, and ease of storage and dosing have been difficult to overcome in a field where cheap and functioning antibiotics are available. For cancer and inflammatory disease there is usually no alternative treatment, which increases the need and demand for a proper treatment and acceptance of, e.g., high costs of treatment. Furthermore, in many programs a lack of predictive animal models has resulted in many disappointments in translating apparent efficacy in animals to humans. In addition, in diseases with large potential markets the vaccine and antibiotic development approach will generally be used. It is therefore time to take a new and revised look into the future to find appropriate niches in infectious diseases where new antibody-based treatments could prove their value and make a major difference. In this, the ability to provide treatments for infections in immunocompromised subjects such as HIV patients or very young or elderly people, as well as treatments for some of the severe potential bioterror infections such as Ebola, could open the eyes of health care providers, researchers, and pharmaceutical developers and provide stepping stones for broader acceptance of the potential for these treatments.

In addition, the general approach to the format and use of these treatments should be reconsidered. The use

of cocktails of multiple antibodies more and more seems to be required to avoid escape mutations and to more efficiently neutralize several toxins or target infection with different mechanisms. Regulatory pathways for developing such cocktails now seem to be in place where mostly only the actual cocktail is tested and only limited additional clinical data demonstrating the contribution from each element is needed. What is also clear is that to generate efficacious antibody treatments, further research into specific mechanisms of pathogenesis is needed, which will assist in choosing the right target antigens. In this respect the vast flexibility that can be achieved by modulating format, effector functions, half-life, and other features provides many opportunities but also challenges in conducting the necessary testing to enable selection of the product with the optimal combination of these features.

Another consideration for the future is to focus more on developing treatments that are adjunct to existing ones, e.g., antibiotic treatment instead of a stand-alone treatment. This might provide that extra efficacy in patients with failing immune response and potentially also compensate for and reduce the development of resistance, thereby ensuring available efficacious treatment options. On the cost side, research into the use of the smaller and cheaper antibody-based fragments could contribute to making antibody-based treatments more attractive and more efficient, and cheaper ways of manufacturing antibodies would contribute to this. In summary, there is no doubt that antibody-based treatments, through their great flexibility to be designed for specific targets on infectious agents or the host immune cells, will and should play a major role in designing new treatments for infectious diseases in the future. Hopefully, several of the programs currently in development will show clinical efficacy and through their approval provide the basis and enthusiasm for this process.

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