REVIEWS

PASSIVE ANTIBODY THERAPY FOR INFECTIOUS DISEASES

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Abstract | Antibody-based therapies are currently undergoing a renaissance. After being developed and then largely abandoned in the twentieth century, many antibody preparations are now in clinical use. However, most of the reagents that are available target non-infectious diseases. Interest in using antibodies to treat infectious diseases is now being fuelled by the wide dissemination of drug-resistant microorganisms, the emergence of new microorganisms, the relative inefficacy of antimicrobial drugs in immunocompromised hosts and the fact that antibody-based therapies are the only means to provide immediate immunity against biological weapons. Given the need for new antimicrobial therapies and many recent technological advances in the field of immunoglobulin research, there is considerable optimism regarding renewed applications of antibody-based therapy for the prevention and treatment of infectious diseases.

HYPERSENSITIVITY REACTION
An inappropriate reaction to an allergen that can be immediate (types I, II and III) or delayed (type IV). Different hypersensitivity reactions involve different antibody classes and effector cells.

ANTIGEN–ANTIBODY COMPLEX DISEASE
An immune complex disease that is caused by the administration of foreign serum or serum proteins, and is characterized by fever, lymphadenopathy and skin welts.

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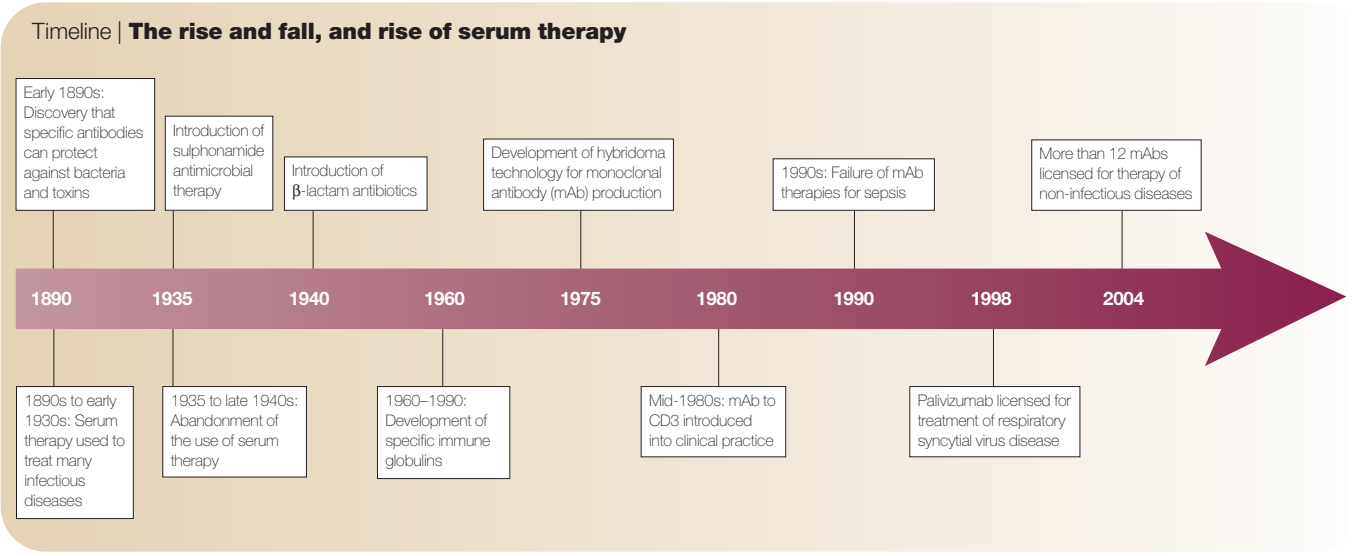
Passive antibody therapy was the first consistently effective antimicrobial strategy. The ability of specific antibodies to protect against bacterial toxins was discovered by Behring and Kitasato in the early 1890s (REF. 1), and this observation led to the rapid development of antibody therapy for the treatment of various infectious diseases[2,3] (TIMELINE). As all antibody preparations were derived from the serum of immunized animals or immune human donors, this form of therapy was known as 'serum therapy'. Serum therapy was effective, but the administration of large amounts of animal proteins was often associated with side effects that ranged from immediate HYPERSENSITIVITY REACTIONS to serum sickness, which is a form of ANTIGEN–ANTIBODY COMPLEX DISEASE. By the 1930s, improvements in antibody purification methods allowed the production of antibody preparations with reduced toxicity, and serum therapy was an effective means of treating many infectious diseases. However, after 1935, the use of serum therapy declined rapidly due to the introduction of sulphonamides and, soon thereafter, other classes of antimicrobial chemotherapy. By the late 1940s, serum was largely abandoned as an antibacterial agent, but antibody-based therapies retained a niche as a treatment for venoms, toxins and certain viral infections.

One of the paradoxes of the history of serum therapy is that its abandonment coincided with advances in antibody purification technology that significantly reduced the toxicity of antibody preparations[4]. One can speculate that these technological advances might have allowed serum therapy to remain competitive with the new antimicrobial agents had the heyday of serum therapy been a decade earlier or had antimicrobial chemotherapy been developed just a few years later. In fact, there were indications at the time that the combination of serum therapy and antimicrobial therapy was effective[3,5]. Unfortunately, the toxicity and complexity of serum therapy was such that the benefits of combined therapy were not sufficient to justify its continued use to treat diseases for which antimicrobial therapy was available. However, in the second half of the twentieth century, the inability to treat certain viral diseases drove efforts to develop antibody preparations derived from immunized human donors for the prophylaxis and treatment of rabies, hepatitis A and B, varicella–zoster virus and pneumonia caused by respiratory syncytial virus (RSV).



A technological revolution

In 1975, the discovery of a method to produce MONOCLONAL ANTIBODIES (mAbs) by immortalizing B cells, which developed into HYBRIDOMA TECHNOLOGY, revolutionized antibody therapeutics[6]. For the first time, it was possible to produce large quantities of an immunoglobulin of a defined specificity and a single ISOTYPE *in vitro*. This innovation allowed the generation of homogeneous antibodies in almost unlimited quantities, eliminating the need for large animal or human donors. Together with the development of new methods for cloning, and recombinant DNA technology, the development of hybridoma technology was supplemented by techniques to genetically modify antibody molecules, including the synthesis of mouse–human chimeric and HUMANIZED ANTIBODIES. In the last decades of the twentieth century, there were several new technological advances, including the immortalization of human peripheral B cells, direct cloning of variable genes into phage expression libraries[7] and the creation of transgenic mice that produce only human antibodies[8]. Although each of these technologies has inherent limitations, together they provide the means to produce mAbs against almost any antigen.

Hybridoma technology was rapidly exploited for clinical use, and a mAb to CD3 was introduced into clinical practice in the mid-1980s to prevent organ rejection. After the introduction of mAbs, there was hope for the rapid development of many therapeutic applications, especially in the field of oncology. However, the pace of discovery and development was slowed by the complexity of successfully targeting tumours and the well-publicized failures of two anti-endotoxin antibodies[9,10]; this failure might have been due to an insufficient understanding of microbial pathogenesis and the mechanisms of antibody action, areas in which studies using mAbs have recently provided a wealth of new information. Nonetheless, by the late 1990s many mAbs were in advanced clinical development. Today, more than twelve mAbs are licensed for therapeutic use, including two that are labelled with radionuclides to deliver tumoricidal radiation (TABLE 1). However, it is striking that only one mAb, palivizumab, has been licensed for an infectious disease (RSV infection), despite the fact that antibodies have proven to be good antimicrobial agents.

Infectious diseases provide a wealth of targets

Passive antibody therapy has been used against many microorganisms that are responsible for human disease, including representatives of the viral, bacterial, fungal and parasitic microbial groups (TABLE 2). In contrast to the use of mAb therapy to treat malignancies, which depends on discriminating between self-antigens that are expressed by normal and tumour cells, passive antibody therapy for infectious diseases is aided by the large antigenic differences between the microorganism and the host. Historically, antibodies have been effective when directed against either microbial antigens or their products, such as toxins. In some microbial diseases, antibodies provide a component of the humoral immune response to natural infection, whereas host defence against other microorganisms relies primarily on cell-mediated immune mechanisms. Nonetheless, there is now considerable evidence to indicate that it is possible to generate mAbs that are protective against microorganisms — such as *Mycobacterium tuberculosis*[11,12], *Listeria monocytogenes*[13], *Leishmania mexicana*[14] and *Histoplasma capsulatum*[15] — for which the activation of humoral immunity is not important for the development of resistance to natural infection. Even intracellular microorganisms can be susceptible to antibodies[16].

In the pre-antibiotic era, antibody therapies were developed against a wide variety of infectious diseases because there were no alternative therapies[3]. Today, although antimicrobial drugs are available, microbial resistance, the emergence of microorganisms that are not susceptible to existing drugs and the fact that antimicrobial drugs are often ineffective in immunocompromised hosts can compromise the efficacy of these drugs. The latter is exemplified by the lack of success in treating the

MONOCLONAL ANTIBODY
A highly specific, purified antibody that is derived from only one clone of cells and which recognizes a single antigen.

HYBRIDOMA TECHNOLOGY
The technology that led to the production of monoclonal antibodies and which involved the generation of an antibody-secreting B-cell line by fusing splenic-derived B cells with an immortal myeloid cell line.

ISOTYPE
The class — or type — of antibody as determined by structural features of the heavy chain constant regions. In humans there are five main isotypes: IgA (2 subclasses), IgD, IgE, IgG (4 subclasses) and IgM.

HUMANIZED ANTIBODIES
Murine monoclonal antibodies are recognized as foreign by the human immune system. To avoid this, humanized antibodies can be constructed in which rodent hypervariable regions (antigen-binding site) are grafted into a human antibody framework.

CD3
A polypeptide complex that is associated with the T-cell receptor and is involved in signal transduction. A CD3-specific monoclonal antibody blocks T-cell activation.

Table 1 | **Monoclonal antibodies licensed for clinical use**

Monoclonal antibody	Use	Year licensed*
Muromonab-CD3	Prevention of organ rejection	1986
Daclizumab	Prevention of organ rejection	1997
Rituximab	Treatment of non-Hodgkin's lymphoma	1997
Abciximab	During cardiac catherization	1997
Trastuzumab	Treatment of breast cancer	1998
Infliximab	Treatment of Crohn's disease	1998
Basiliximab	Prevention of organ rejection	1998
Palivizumab	Prophylaxis of RSV disease	1998
Alemtuzumab	Treatment of chronic lymphocytic leukaemia	2001
Adalimumab	Treatment of rheumatoid arthritis	2002
Ibritumomab-tiuxetan-^{90}Y‡	Treatment of lymphoma	2002
Tositumomab/Tositumomab-^{131}I‡	Treatment of lymphoma	2003
Omalizumab	Treatment of asthma	2003
Cetuximab	Treatment of colon cancer	2004
Bevacizumab	Treatment of colon cancer	2004

*Licensed in the United States. Information obtained from the US Food and Drug Administration. ‡Ibritumomab-tiuxetan-^{90}Y and Tositumomab/Tositumomab-^{131}I are forms of radioimmunotherapy known by the trade names Zevalin and Bexxar, respectively. RSV, respiratory syncytial virus.

infectious diseases that arise in the setting of severe immunosuppression, such as bone marrow and organ transplantation, and AIDS. Interestingly, on the basis of evidence that mechanisms of antibody efficacy can include the regulation or induction of cellular immune responses[17], antibody therapy with or without additional immunomodulators might have promise for treating infectious diseases in immunocompromised hosts. So, antibodies represent a new, although historically validated, approach to the development of therapies against microorganisms that cause disease in individuals with impaired immunity and/or for which there are no available drugs.

Antibody-based therapies: pros and cons

The advantages and disadvantages of antibody-based therapies are often compared with those of conventional antimicrobial drugs. However, immunoglobulins are sufficiently different in their physical characteristics and modes of action to be regarded as a distinct therapeutic class.

Advantages. Antibody-based therapies that use human or humanized antibodies have low toxicities and high specificities. The high specificity of antibodies is both an advantage and a disadvantage. The advantage of high specificity is that antibody-based therapies target only the microorganism that causes disease and, therefore, should not alter the host flora or select for resistance among non-targeted microorganisms. However, high specificity also means that more than one antibody preparation might be required to target microorganisms with high antigenic variation. In fact, in the case of serum therapy, numerous type-specific sera were developed for the treatment of pneumococcal pneumonia because only type-specific sera were effective against pneumococci[2,5]. In theory, a disadvantage of high specificity is the emergence of variants that lack the determinant that the antibody recognizes, such as viral escape mutants. The use of cocktails of antibodies that are specific for several antigens could obviate this concern. However, this approach would also have the drawback of increasing the cost of production and the complexity of regulatory issues involving efficacy and safety.

The high specificity of antibody molecules is complemented by their versatility, which allows an antibody that binds a single determinant to mediate various different biological effects (FIG. 1). As natural products of the immune system, antibodies can interact with other immune components. Some mechanisms of antibody action, such as toxin and virus neutralization and complement activation, and direct antimicrobial functions, such as the generation of oxidants, are independent of other host immune components. By contrast, antibody-dependent cellular cytotoxicity and opsonization are dependent on cellular and other host mediators. In recent years, the recognition that antibodies can be immunomodulators, bridging the innate, acquired, cellular and humoral immune responses, has revealed new mechanisms of antibody-mediated immunity and has provided a better understanding of how and why antibodies are effective against microorganisms for which they do not mediate a direct biological effect[17]. In fact, there is evidence that B cells and antibodies can protect against certain infectious diseases by reducing host damage resulting from the inflammatory response[17]. This could partly explain the efficacy of intravenous immunoglobulin, which is used to treat certain inflammatory conditions. Evidence that specific immunoglobulin G (IgG)–Fc receptor interactions can inhibit the inflammatory response[18] indicates that antibody therapy could be effective against certain infectious diseases by reducing the damage that results from the host inflammatory response.

Table 2 | **Microorganisms against which antibody has been used to target human diseases***

Microorganism	Disease in humans	References
Bacillus anthracis	Anthrax	50
Bordetella pertussis	Whooping cough	51
Clostridium tetani	Tetanus	52
Clostridium botulinum	Botulism	53
Cryptococcus neoformans	Cryptococcosis	54
Cryptosporidium parvum	Cryptosporidiosis	55
Enterovirus	Gastrointestinal-tract infections	56
Group A streptococci	Several illnesses including sore throats, necrotizing fasciitis	57
Hepatitis B virus	Hepatitis B	58
Measles virus	Measles	59
Mycobacterium tuberculosis	Tuberculosis	60
Neisseria meningitidis	Meningitis	2,61
Parvovirus	Aplastic anaemia	62
Rabies virus	Rabies	63
Respiratory syncytial virus (RSV)	RSV infection	64
Streptococcus pneumoniae	Pneumonia	2
Varicella–zoster virus	Shingles, chickenpox, pneumonia	65
Variola major	Smallpox	66

*This is not a complete list.

In addition, the use of antibodies as therapeutic reagents has the advantage that there are several isotypes, which can function therapeutically in either an intact form or as fragments. In the intact molecule, the variable region (Fab) binds antigen, whereas the constant region (Fc) determines the biological properties of the immunoglobulin molecule, such as serum half-life, interaction with cellular Fc receptors and the ability to activate complement. When the binding of antibody to a target antigen is sufficient to mediate an effect, which can occur when an antibody is functioning as an antitoxin or antiviral agent, an antibody fragment can be sufficient for efficacy. However, when antibody efficacy is dependent on immunomodulation or interaction with effector cells to mediate phagocytosis, complement activation or antibody-dependent cellular cytotoxicity, an intact immunoglobulin molecule is required for efficacy. Whether a Fab fragment or intact antibody is suitable as a therapeutic agent also depends on the microorganism that is being targeted and the immunological status of the host. As biological effects that depend on the Fc receptor could require intact host immunological function, antibodies that have direct antimicrobial effects or that mediate beneficial effects by the binding of Fab alone might be more useful in immunocompromised hosts. Antibodies with direct antimicrobial properties have recently been described against several microorganisms, including *Borrelia* spp.[19], *Candida albicans*[20,21] and *Cryptococcus neoformans*[22].

An important potential advantage of antibody therapies is that they can be synergistic or additive when combined with conventional antimicrobial chemotherapy against bacterial and viral diseases (reviewed in REFS 3,5). In addition, recent studies suggest that combinations of antibodies and drugs are more effective against fungal infections than when either therapy is used alone[15,20,23]. Consequently, antibody-based therapies could easily be incorporated into existing treatment protocols; however, demonstrating the advantages of combination therapy in rigorous clinical trials can be logistically and practically difficult, and the use of combination therapy would be more expensive.

In addition to their advantages as therapeutic agents, antibodies have had a central role in vaccine development. Historically, vaccine development for numerous infectious diseases was fuelled by antibody-based therapies and research into antibody-mediated immunity. Antibody therapy can be protective against an infectious disease, which suggests that a vaccine that elicits similar antibodies could be protective against the relevant pathogen. For example, successful passive antibody therapy against pneumococcal pneumonia and diphtheria preceded the development of vaccines against these diseases. More recently, the generation of protective mAbs against *C. neoformans* and *C. albicans* identified polysaccharide antigens that were then used to design effective conjugate vaccines[24,25]. Protective antibodies to microbial polysaccharides can be used to identify PEPTIDE MIMOTOPES that elicit protective antibody responses[26], and antibodies that elicit protective ANTI-IDIOTYPIC RESPONSES can be used directly as immunogens[27]. Efforts to develop antibody-based therapies can, therefore, promote vaccine development.

Disadvantages. As antibodies are natural products they must be produced in cell lines or other live expression systems. This raises the theoretical concern that there could be contamination of antibody preparations by

PEPTIDE MIMOTOPES
Peptides that mimic natural epitopes.

ANTI-IDIOTYPIC RESPONSES
The antigen-binding site of an antibody is also known as the idiotype. An antibody response to this region can generate antibodies that bear the image of the original immunogen or antigen.

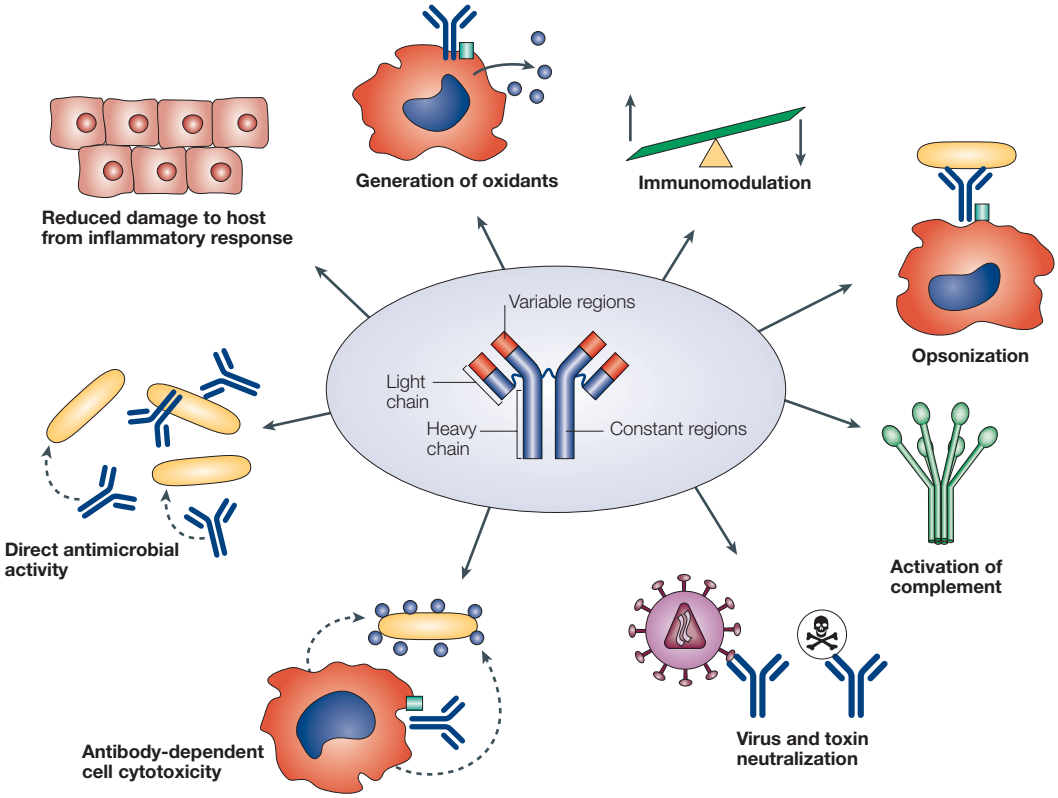


Figure 1 | **The different biological effects of antibodies.** Toxin and virus neutralization, complement activation and direct antimicrobial functions such as the generation of oxidants are independent of other components of the host immune system, whereas antibody-dependent cellular cytotoxicity and opsonization depend on other host cells and mediators.

infectious agents such as prions or viruses. Although tight regulation and regulatory vigilance and surveillance can reduce this concern, the need for ongoing monitoring and testing for contamination contributes to the high cost of developing and administering antibody therapies. In addition, antibody-based therapies require considerable logistical support. As antibodies are proteins, they cannot be given orally, except for those used to treat certain types of mucosal infectious diseases, such as *Cryptosporidium parvum*-associated diarrhoea, and therefore, systemic administration is required.

Owing to their high specificity, antibodies have activity against the microorganism to which they bind. Antibody therapy therefore requires knowledge of the causative microbial agent, which in turn requires rapid microbiological diagnosis. Additionally, because antibody efficacy is highest when given early in the course of infection, rapid diagnosis is essential for the success of antibody therapy. For example, the efficacy of serum therapy for pneumococcal pneumonia is markedly reduced after the first three days of symptoms[2,5]. In the first decades of the antibiotic era, the lack of innovation in microbiological diagnosis was tolerated owing to the availability of broad-spectrum antimicrobial agents. However, the need for rapid diagnostic techniques has assumed greater urgency with the emergence of fungi in immunocompromised hosts and nosocomial infections, resistant bacteria and previously unknown viral diseases for which the available antimicrobial armamentarium is inadequate. At the same time, the development of PCR and other rapid diagnostic techniques has provided new options that could support antibody-based therapies. Importantly, the efficacy of anti-infective antibody-based therapies can be assessed relatively easily, as there are well-defined clinical end points that can be used to determine whether therapy has been successful.

A peculiar characteristic of antibody-based therapies is that their efficacy diminishes rapidly as the duration of infection increases. Antibody reagents with therapeutic potential are often evaluated by administration to naive hosts before infection. Although this approach to select antibody preparations was well established during the development of serum therapy, it is noteworthy that serum was effective in humans even when administered several days after the onset of symptoms, despite having little or no therapeutic efficacy in mouse models[2]. However, even in humans, the efficacy of therapeutic antibodies diminished rapidly after the onset of symptoms[2,5]. The mechanism responsible for this is not well understood, but might reflect a rapid increase in the microbial burden in the animal models used, which are usually selected on the basis of their marked susceptibility to the agent in question[28]. A loss of efficacy with increased duration of infection or disease could limit the application of antibody-based strategies to prophylaxis and/or conditions where an early diagnosis is possible.

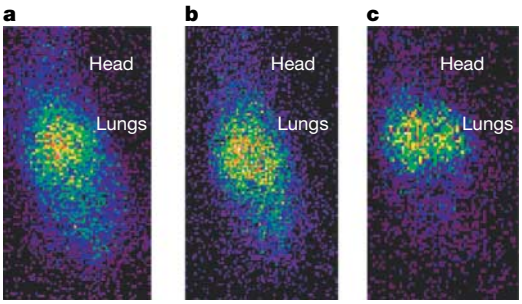


Figure 2 | ***Cryptococcus neoformans* infection and radioimmunotherapy.** Biological distribution of ^{111}Indium-labelled whole 18B7 monoclonal antibody (mAb) (**a**), F(ab)2 (**b**) and Fab (**c**) 24 h post-injection. AJ/Cr mice were infected intravenously with 10^5 *C. neoformans* cells 24 h before injection with radiolabelled mAbs. The radiation localizes to the lungs, which are heavily infected with *C. neoformans*. Activity in the lungs is seen for all three carriers. For methodology, see REF. 35.

One of the greatest advantages of antibody therapy, namely high specificity, means that the potential market for a reagent is likely to be small — as the size of the market is proportional to the number of affected individuals. Given the large expenses that are associated with drug discovery and development, it is likely that development of antibody-based therapies will focus mainly on infectious diseases that are sufficiently common to provide financial rewards. In practice, this means that, although antibody therapy could be effective, such therapy is unlikely to be developed for relatively rare infectious diseases because the costs are considered prohibitive.

The high costs of production, storage and administration of antibodies are disadvantages of antibody-based therapies. For example, in the United Kingdom, the cost of palivizumab therapy for the prevention of RSV disease is estimated at UK £2,500, which affects the cost/benefit ratio[29]. In the field of infectious diseases, discussion of the costs of antibody therapy is often affected by the fact that they are compared with relatively cheap antimicrobial drugs. However, a true comparison of the costs must include the fact that nonspecific drug therapy selects for resistant organisms and predisposes individuals to super-infection, which in turn incurs additional costs for prolonged hospitalization, therapy and patient follow-up. As the high specificity of antibody therapies makes it unlikely that they will select for resistance in non-targeted microorganisms, they should not markedly impact on the resident microflora. So, high costs could be offset by lower levels of resistance and fewer nosocomial infections. The cost of antibody development notwithstanding, it is notable that the time to development of a potential antibody therapy, provided an appropriate antigen is available, is considerably shorter than that needed to develop a vaccine[30].

New directions in anti-infective antibody therapy

A great advantage of antibody-based anti-infective therapies is their inherent flexibility — in addition to the availability of nine natural isotypes with different half-lives, the ability to activate complement and the ability to interact with different Fc receptors, it is also possible to modify immunoglobulins to have new antimicrobial capabilities. One strategy is to target infected host cells by linking cellular toxins to antibodies against microbial antigens that are expressed on the surfaces of host cells. Along these lines, antibodies to murine cytomegalovirus that are linked to a deglycosylated ricin A chain have been shown to target cytomegalovirus-infected cells[31]. Similarly, viral envelope proteins that are expressed on the surface of HIV-infected, virus-synthesizing cells can be targeted with antibodies linked to the ricin A chain[32] or *Pseudomonas* spp. exotoxin A[33]. Immunotoxins are particularly attractive for the therapy of infectious diseases in which the pathogen is intracellular and uses this environment to reproduce. However, they are not necessarily active against extracellular microorganisms as the antibodies must be internalized relatively rapidly and the covalent attachment of a toxin to the immunoglobulin molecule has the potential to elicit an antibody response in treated hosts, which would limit repeated use.

Another strategy for antibody targeting is to link radionuclides to specific antibodies such that the immunoglobulin molecule targets and delivers microbicidal radiation to the microorganism (FIG. 2). This approach is known as radioimmunotherapy and has been successfully used in cancer treatment[34]. A proof-of-principle for the use of this strategy to treat an infection was established by demonstrating that mAbs to the *C. neoformans* capsular glucuronoxylomannan labelled with ^{213}Bi or ^{188}Re could be used to treat murine cryptococcosis[35]. The administration of radiolabelled mAbs prolonged survival and reduced the organ fungal burden in this model, whereas an irrelevant radiolabelled mAb or unlabelled specific mAb had no effect[35]. Apart from a transient drop in serum platelet counts, no measurable toxicity was detected in mice that were treated with radiolabelled mAbs[36]. The efficacy of radioimmunotherapy against murine pneumococcal infections has also been established, showing the applicability of this approach to a bacterium with a fast doubling time[37]. Analysis of the susceptibility of fungal cells to radiolabelled mAbs that bind to surface antigens *in vitro*, using both *C. neoformans* and *H. capsulatum*, showed markedly greater susceptibility to killing by antibody-delivered particulate (β- and α-particles) radiation than to external γ-radiation[38]. Although this is not well understood, it is possible that particulate radiation that is delivered in close proximity to microbial cells has greater killing power than γ-photons. Alternatively, antibody effects, such as the recently described ability to generate oxygen-related oxidants[39], might synergistically increase the killing power of locally emitted radiation.

Attaching a radionuclide to an immunoglobulin converts the antibody into a microbicidal molecule, even if the antibody is not protective independently. Methods have been developed for the stable attachment of radionuclides to immunoglobulins such that *in vivo* hydrolysis is not a major problem[40]. Consequently, radiolabelling has the potential to enhance the power of passive antibody therapy by

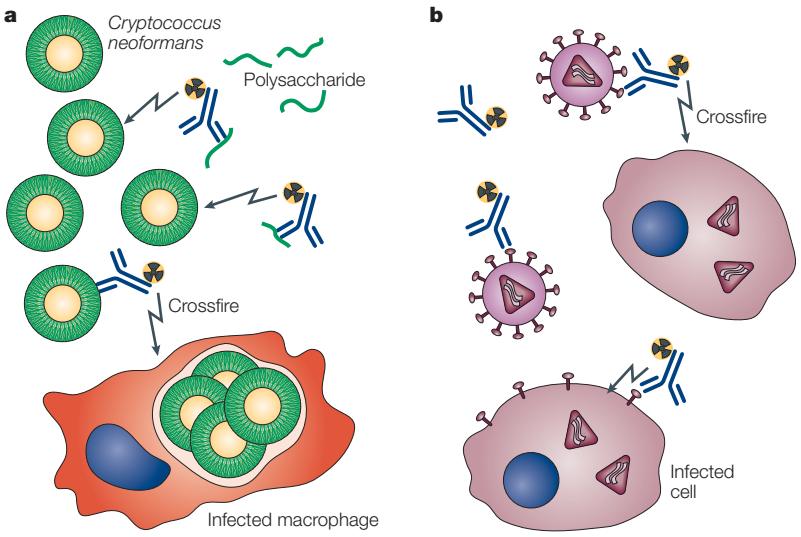


Figure 3 | **A schematic representation of the mechanisms by which radioimmunotherapy is effective against microorganisms. a** | For *Cryptococcus neoformans* infection, a radiolabelled antibody binds to both capsular and shed polysaccharide, and results in localized fungicidal radiation. Macrophages infected with replicating intracellular fungi can also be killed by a crossfire effect. **b** | The proposed mechanism by which radiolabelled antibody is effective against an intracellular pathogen such as a virus that expresses microbial antigens on the cell surface after cellular infection.

conferring the power to kill the targeted microorganism on any specific antibody. Therefore, radioimmunotherapy for infectious diseases should theoretically be effective in immunocompromised hosts and might also be effective against chronically infected cells that express microbial antigens on their surface (FIG. 3), which could be a powerful means to eliminate latent microorganisms that might be harboured by cells and that avoid host defence mechanisms. As particulate radiation also kills infected cells through a 'crossfire' effect (radiation emanating from a cell hits an adjacent or a distant cell), not every microbial cell in the infected area needs to be bound by a labelled antibody molecule to be killed. In contrast to immunotoxins, radiolabelled human antibodies do not need to be internalized, are unlikely to elicit significant immune responses that would limit subsequent use and the unlikely separation of the chelator–radiometal label would not produce a toxic product. However, the application of this technology to infectious diseases is in its infancy, and the extent of its usefulness and potential toxicity remain to be defined.

Another approach to confer additional biological properties to an immunoglobulin is to create a bispecific antibody in which one arm of the Fab fragment recognizes a microbial epitope, and the other recognizes a host immune component, which is often a relevant receptor. Numerous reports of bispecific antibodies with effective antimicrobial action have been published. For example, bispecific antibodies consisting of a pathogen-binding Fab and a complement-receptor-binding Fab have been shown to be effective in promoting the clearance of bacteriophage[41] and *Pseudomonas aeruginosa*[42].

PROZONE EFFECT
A decrease in an antigen–antibody reaction that occurs as the concentration of antibody or antigen increases.

Unsolved problems in antibody therapy

After more than 110 years of use in humans, there are still many unsolved problems that limit the widespread application of antibody-based therapies. The development of therapeutic antibodies remains, for the most part, an empirical science. For many infectious diseases, the current understanding of microbial pathogenesis is insufficient to predict the microbial antigens against which therapeutic antibodies should be raised. In addition, current immunological knowledge is insufficient to predict which antibodies are effective against specific microorganisms, particularly in immunocompromised hosts. The relationship between antibody isotype and efficacy is unclear for many microorganisms, and generalizations are difficult. For example, murine IgG3 mAbs have been shown to be protective against *Streptococcus pneumoniae*[43] and *M. tuberculosis*[11], but are relatively ineffective against *C. neoformans*[44]. Human IgM has been found to be highly protective against experimental *C. neoformans* and *S. pneumoniae* infections[45,46], although in vaccine development the presence of specific serum IgG is used as a surrogate for immunity. However, we do not know if insights into isotype function that have been gained from animal studies can be applied to humans. It is clear that antibody binding to certain epitopes on a given antigen can result in protection, whereas binding to other epitopes is ineffective, but we cannot currently predict which epitopes elicit protective antibodies. Another difficult problem is estimating the amount of antibody to use for therapy. Administration of too little antibody can produce no therapeutic effect, whereas administration of too much antibody can produce disconcerting PROZONE-like effects, whereby antibody efficacy is lost and antibody administration can be detrimental to the host[47–49]. This seems to be a result of an excess of antibody interfering with the host microbicidal mechanisms and changes in cytokine expression[47,48].

The near and far horizons

Passive antibody administration is currently used to treat and prevent diseases caused by hepatitis B virus, rabies virus, RSV, *Clostridium tetani*, *Clostridium botulinum*, vaccinia virus, echovirus and enterovirus. Antibody therapies against HIV, rotavirus, bacterial sepsis, cytomegalovirus, *C. neoformans* and *C. albicans* are in clinical development. Furthermore, there are many monoclonal antibodies against infectious diseases in advanced preclinical development, and one can confidently expect that many more antibodies will be developed for clinical use. In this regard, the realization that passive antibody therapy can provide immediate immunity against biological weapons has spurred the search for, and development of, protective antibodies against many selected agents including *Bacillus anthracis* toxins, Ebola virus and the *C. botulinum* toxins. Consequently, current efforts to develop countermeasures to biological weapons could be an important stimulus for the development of antibody therapies for infectious diseases. However, the combination of

manufacturing and economic hurdles, the need for a cold chain, intravenous administration, rapid diagnosis and pathogen specificity, and the continuing availability of antimicrobial drugs indicates that the development of anti-infective antibody therapies will progress slowly and will almost certainly lag behind the application of antibody therapies to non-infectious diseases for which no therapy is available and where the potential market is larger. Nevertheless, we predict that, in the future, the use of this proven antimicrobial strategy will increase, and anticipate a time when antibody therapy, antimicrobial chemotherapy and possibly other forms of immunotherapy are used in combination to treat a wide variety of infectious diseases.

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Competing interests statement

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