CHAPTER 9

The Antigenome:
From Protein Subunit Vaccines to Antibody Treatments of Bacterial Infections?

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Abstract

New strategies are needed to master infectious diseases. The so-called "passive vaccination", i.e., prevention and treatment with specific antibodies, has a proven record and potential in the management of infections and entered the medical arena more than 100 years ago. Progress in the identification of specific antigens has become the hallmark in the development of novel subunit vaccines that often contain only a single immunogen, frequently proteins, derived from the microbe in order to induce protective immunity. On the other hand, the monoclonal antibody technology has enabled biotechnology to produce antibody species in unlimited quantities and at reasonable costs that are more or less identical to their human counterparts and bind with high affinity to only one specific site of a given antigen. Although, this technology has provided a robust platform for launching novel and successful treatments against a variety of devastating diseases, it is up till now only exceptionally employed in therapy of infectious diseases. Monoclonal antibodies engaged in the treatment of specific cancers seem to work by a dual mode; they mark the cancerous cells for decontamination by the immune system, but also block a function that intervenes with cell growth. The availability of the entire genome sequence of pathogens has strongly facilitated the identification of highly specific protein antigens that are suitable targets for neutralizing antibodies, but also often seem to play an important role in the microbe's life cycle. Thus, the growing repertoire of well-characterized protein antigens will open the perspective to develop monoclonal antibodies against bacterial infections, at least as last resort treatment, when vaccination and antibiotics are no options for prevention or therapy. In the following chapter we describe and compare various technologies regarding the identification of suitable target antigens and the foundation of cognate monoclonal antibodies and discuss their possible applications in the treatment of bacterial infections together with an overview of current efforts.

Introduction

Infectious diseases remain a major threat against human life. Microbial infections are still out of control in many parts of the less developed world where they count for most of the deaths, but also cause an often underestimated toll of death (e.g., community acquired Pneumococcal diseases and *Pseudomonas* infections in patients in intensive care), life-long mutilation (infertility due to *Chlamydia trachomatis*), medical complication due to nosocomial infections caused most often by *Staphylococcus aureus, Enterococcus faecalis, Klebsiella* ssp and fungi. It is estimated that nosocomial infections annually add US$5-10 billion to the cost of the national healthcare system in the United States.[1] Apart from infections caused by viruses and protozoa that only in specific instances can

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be treated with suitable pharmaceuticals, the emergence of antibiotic-resistant strains of nearly all kinds of bacterial pathogens in the community and in hospitals is occurring at an increasingly alarming rate.[2,3] The increase of nosocomial infections, the comeback of bacterial infections in immune suppressed individuals, e.g., TB in AIDS patients,[4] and the lately appeared scenario of bio-terrorism, e.g., in the context of anthrax,[5,6] are reminders that new strategies are needed to master infectious diseases in prophylactic and therapeutic settings.

Vaccination is undeniable the most successful medical intervention in the control of infectious diseases. However, since vaccine-induced immune protection against specific microbes takes more than a couple of weeks to develop and postexposure vaccination is only exceptionally a useful tool, combination with passive immunization is indispensable (e.g., treatment against the rabies virus, reviewed in ref. 7), when instant protection or treatment is required. Therapeutic vaccines are still in the exploratory stage of development and more prone to find their application in the treatment of chronic infectious diseases,[8,9] rather than to become an immediate measure against a sudden infectious threat. On the other hand, most vaccines seem to confer protective immunity to the vaccinated individuals by the means to induce specific antibodies that capture the invading microbe, prior it had an opportunity to colonize in the exposed host. The so-called "passive vaccination", i.e., prevention and treatment with specific antibodies, has a proven record and potential in the management of infections.

Already the pioneers of early microbiology and immunology in the late 19th century, led by their prominent proponents, Emile Roux and Emil von Behring, have realized the concept of "passive vaccination", namely that sheep and horses inoculated with filterable toxin extracts derived from *Corynebacterium diphteriae* cultures were able to mount an "anti-toxin" in their blood. Serum derived from the animals' blood was able to rescue children in the lethal stage of the infection caused by the same pathogen. Revisiting this historical landmark therapy of diphtheria, it was realized that the "anti-toxin" in the serum of inoculated animals is synonymous with a protein species coined today antibodies and the "toxin" with a virulence factor secreted by the pathogen during infection. Thus, the remarkable and groundbreaking therapy concept explored more than 100 years ago, has paved the way to "passive immunization", i.e., all kind of serum-treatments that have found their broad medical applications in prevention of e.g., viral infections or in emergency treatments against e.g., snake venoms.[10,11] Serum antibodies against microbes and even isolated antigens, like the diphtheria toxin, are polyclonal, meaning that they bind—in case of a specific antigen molecule—to a variety of sites or—in case of a microbe—to multiple surface structures.

The advent of the monoclonal antibody technology launched by Georg Kohler and Cesar Milstein nearly 30 years ago, has enabled biotechnology to engineer specific antibody species that bind with high affinity to only one specific site of a given antigen and can be produced in unlimited quantities. Follow-up technologies made it possible to produce monoclonal antibodies that are more or less identical to their human counterparts, employing microbial and tissue culture resources for manufacturing.[12] During the last decade, monoclonal antibodies have infiltrated the therapeutic arena with great success and thereby provided a plethora of novel treatments against a variety of typically devastating diseases including specific cancers, autoimmune diseases and other pathological conditions.[13] The common denominator of all monoclonal antibodies used in therapy is to bind to highly specific sites of typically well characterized protein targets and thereby intervene with biological functions involved in the pathogenic condition; e.g., to growth hormone receptors expressed at the surface of malignant cells.[14,15] Interestingly, monoclonal antibodies engaged in the treatment of specific cancers seem to work by a dual mode; they mark the cancerous cells for decontamination by the immune system, but also block a function that intervenes with cell growth.[16]

Progress in the identification of specific antigens has become the hallmark in the development of novel subunit vaccines that only contain single specific structures derived from the microbe in order to induce protective immunity. The first viral subunit vaccine on the market that has become a great success is directed against Hepatitis B virus and based on recombinant protein

technology. Also pathogen-specific glycosides coupled to carrier proteins are successfully used in so-called conjugated vaccines directed against bacterial infections; an example is "Prevnar" a registered vaccine against *Pneumococcus*.[17] The successful development of subunit vaccines comprising isolated microbial components as antigens has supported the notion that antibodies per se, may suffice to neutralize pathogens in the body even in a setting of "passive vaccination". So far only one anti-infective monoclonal antibody, which is directed against the Respiratory Syncytial Virus (RSV) (Palivizumab), has entered the therapeutic arena.[18] A number of anti-infective antibodies based on specific antigens against bacterial infections are in the stage of clinical and preclinical development (Table 1).

The availability of the entire genome sequence of pathogens and subsequently the application of proteome and genome based technologies have facilitated the identification of highly specific protein antigens suited for the development of novel bacterial subunit vaccines.[19] One of the recently described methods designed to comprehensively mine bacterial genomes for protective antigens, has taken advantage of antibodies derived from humans who have encountered the target pathogen with positive outcome. The sum of all protein antigens that are recognized by cognate antibodies from individuals exposed to the pathogen has been defined as antigenome.[20-22] Typically the antigenome comprises 100 to 200 antigens. Applying a number of selective filters and criteria to the antigenome, in vitro validation makes it possible to reduce the number of best-suitable candidate antigens for vaccine development to about 15 to 30 (unpublished data). Such antigens are presently tested in advanced preclinical and early clinical trials (Kuklin et al[23] and unpublished data). The availability of bacterial protein antigens with promising profiles for vaccine design, but also the identification of specific host targets, have provided novel gates to develop monoclonal antibodies for protection and treatment against specific infectious diseases. In the following chapter we will discuss the impact of discovery and characterization of specific antigens on the development of novel vaccines and antibody treatments.

A New Paradigm in Bacterial Vaccine Development

The capability of the human immune system to identify and eliminate pathogens and pathogen-infected cells is the cornerstone of immunization, the most effective strategy to prevent infectious disease. However, vaccines are still not available against major pathogens including *Meningococcus* serogroup B, *Gonococcus*, *Helicobacter pylori* and *Shigella*. Traditional vaccines are mainly based on inactivated or attenuated microbes or more recently on polysaccharides of a particular pathogen. Due to the fact that such vaccines cannot prevent numerous diseases, or even worse, induce severe side effects, novel and defined vaccines are being developed to overcome these limitations. Improved vaccines are needed to combat diseases for which current vaccines are inadequate (e.g., tuberculosis) or against pathogens that had not been on the target list for immunization, such as *Staphylococci* and *Enterococci* both with an enormous potential to develop drug resistance.[24,25] The recently emerging threat of bioterrorism boosts the need for new vaccines further.

Most of the new generation vaccines comprise subunits of pathogens (purified protein, toxoid, polysaccharide with or without conjugation) and have made major headways in controlling serious diseases. At present, there are only two vaccines based on recombinant proteins (against Hepatitis B and Lyme disease) that are shown to be effective in preventing human infections. Nevertheless, protein based recombinant vaccines are considered to be the most promising approach to meet the demands of future vaccinology.

In order to design novel subunit vaccines, the proper antigens have to be identified and subsequently evaluated in experimental animal models mimicking human diseases. While vaccine development for obligate pathogens with well-defined virulence mechanisms has progressed well, those bacteria that are in the focus of current vaccine efforts (e.g., opportunistic pathogens and those with multiple serotypes) have more complex pathogenesis.

Vaccinologists are witnessing a remarkable revolution in technologies that now contribute to rapid identification of novel vaccine components against many important human pathogens.

Table 1. Therapeutic antibody approaches against bacterial infections

Drug	Pathogen	Antigen Target	Type of Antibody	Highest Phase	Originator	Indication
Aurograb®	S. aureus	ABC transporter	Human-derived single chain variable fragment (scFv) therapeutic antibody	III	NeuTec Pharma	Methicillin-resistant S. aureus infections
Altastaph™	S. aureus	S. aureus Type 5 and Type 8 polysaccharides	Polyclonal (5% Ig prepared from donors immunized with StaphVAX™)	II	Nabi Biopharmaceuticals	Staphylococcal infections
Pagibaximab	Staphylococcus spp.	Lipoteichoic acid (LTA) S. epidermidis	Chimeric mAb	II	Eli Lilly/Biosynexus	Staphylococcal infections
Aurexis®	S. aureus	S. aureus ClfA	Humanised mAb	II	Inhibitex	Treatment—in combination with standard-of-care antibiotics—of serious S. aureus infections in hospitalized patients
Veronate®	S. aureus	Staphylococcal fibrinogen-binding proteins SdrG and ClfA (MSCRAMM)	Human polyclonal	Preregistration	Inhibitex	Staphylococcal infections in VLBW (very low birth weight) infants: Prevention of hospital-associated infections in premature infants weighing less than 1,250 grams

Table 1. Continued

Drug	Pathogen	Antigen Target	Type of Antibody	Highest Phase	Originator	Indication
ETI 211	S. aureus	S. aureus protein A	Antibody conjugate: anti-human complement receptor Type-1 mAb chemically cross-linked with an anti-S. aureus protein A mAb	Preclinical	Elusys Therapeutics	Methicillin-resistant S. aureus infections
S. aureus mAb	S. aureus	IsdB	Fully human mAbs	Preclinical	Merck/Intercell	Staphylococcal infections
SdrG mAb	S. epidermidis	SdrG-fibrinogen-binding MSCRAMM protein	Human polyclonal	Preclinical	Inhibitex	S. epidermidis infections
Enterococcal mAb	Enterococcus	MSCRAMM® proteins	Fully human mAbs	Preclinical	Inhibitex/Dyax	Drug-resistant enterococcal infections
IC 47 therapeutic antibodies	S. pneumoniae	Surface proteins	Fully human mAbs	Preclinical	Kirin/Intercell	Pneumococcal infections in immunocompromised patients
KBPA 101	P. aeruginosa	Directed against pseudomonal serotypes	Fully human mAbs	II	Kenta Biotech	Pseudomonal infections
Anti-P. aeruginosa mAbs	P. aeruginosa	Natural human immune response	Fully human mAbs	I	Berna Biotech	Pseudomonal infections
Anti-P. aeruginosa mAbs	P. aeruginosa	Undisclosed	Fully human mAbs	Preclinical	Millenium Biologix Corporation	Pseudomonal infections

Table 1. Continued

Drug	Pathogen	Antigen Target	Type of Antibody	Highest Phase	Originator	Indication
MDX 066	*Clostirium difficile*	Toxin A	Fully human mAbs	II	Medarex, University of Massachusetts Medical School	*C. difficile* infections, Diarrhea
MDX 1388	*C. difficile*	Toxin B	Undisclosed	Preclinical	Medarex, MBL	*C. difficile* infections
DiffCAM™	*C. difficile*	*C. difficile* toxins	Bovine immunoglobulins	I	ImmuCell	*C. difficile* infections
Anti-botulism neurotoxin mAbs	*Clostridium botulinum*	Type A-botulinum neurotoxin	mAbs	Preclinical	XOMA	Biological agents used in bioterrorism
Urtoxazumab	Shiga-like toxin-producing *E. coli*	B-subunit of shiga-like toxin II (SLT II)	Recombinant humanised mAbs	II	Teijin Pharma	haemolytic uraemic syndrome
Raxibacumab	*B. anthracis*	*B. anthracis* protective antigen	Fully human mAbs	I	Human Genome Sciences	Anthrax
Valortim™	*B. anthracis*	*B. anthracis* protective antigen	Fully human mAbs	I	Medarex	Anthrax
Anthim™	*B. anthracis*	*B. anthracis* protective antigen	Heteropolymer chemically linked mAb	I	Elusys Therapeutics	Anthrax
Afelimomab	Septic shock	human TNFα	Murine mAbs	III	Abbott GmbH & Co. KG	septic shock
Anti-TNFα	Septic shock (among others)	human TNFα	Sheep polyclonal	II	Protherics	septic shock (among others)

Data were collected from the Adis R&D Insight database and homepages of listed companies in July 2006 using the world wide web. Antibodies were sorted according to antigen target: first, antibodies targeting pathogen surface structures are listed, followed by anti-toxin antibodies and last antibodies against TNFα. Monoclonal Antibodies are abbreviated with mAbs. This is not a complete list.

The availability of complete genome sequences of pathogens has dramatically changed the perspectives for developing improved and novel vaccines by increasing the speed of target identification. Genomics-based technologies have many advantages compared to conventional approaches, which are time-consuming and usually identify only abundant antigens expressible under in vitro culture conditions. Strategies based on genomics have made major contributions to the identification and selection of novel vaccine candidates to combat bacterial infections by exploiting genome sequence information in alliance with adjunct technologies, including in silico prediction (bioinformatics), expression analyses (random mutagenesis, microarrays, in vivo expression technologies), or protein/peptide based selection methods (proteomics and immuno-selection using peptide expression libraries). Although, most technologies can be readily applied to most pathogens, certain strategies are more suitable than others due to distinct advantages and limitations.

The most promising candidate antigens have to be (1) expressed during human disease; (2) accessible (surface bound or secreted) for functional antibodies or effector immune cells; (3) conserved among strains; (4) essential for in vivo survival in order to avoid counter selection; and (5) protective in animal models mimicking the relevant human disease. There is no technology available today that can select antigen candidates fulfilling all five attributes. However, a comprehensive selection procedure meeting the key criteria can be combined with a validation screening that addresses the remaining requirements.

To date, approximately 300 pathogen genome sequences have been determined (http://www.tigr.org/cmr). Genome sequences of bacterial pathogens contain an average of 2700 genes, thus appropriate selection criteria have to be applied to reduce the number of antigen candidates for empirical testing. Bioinformatics has been successfully employed for the prediction of candidate antigens of extracellular pathogens, due to the specific features easing the prediction of cell surface and secreted proteins and/or the identification of genes that show sequence and/or structural homology to known virulence factors.[26] This type of genome-based systematic search for vaccine candidates was termed "reverse vaccinology". The validity of this approach was first confirmed by the identification of protective antigens from *Meningococcus* serogroup B and later from *Pneumococcus* (reviewed in ref. 27). "Reverse vaccinology in silico prediction" typically targets up to 25% of all genome-encoded proteins and, thus, necessitates subsequent high through-put cloning and recombinant protein expression. Inclusion of more restrictive selection criteria became possible through the availability of several genomes for individual pathogenic species. Comparative genomics is another suitable tool to identify genes shared among species of related pathogens or, alternatively, to identify genes present only in pathogenic, but not in attenuated or naturally nonpathogenic strains or species. Such approaches have been successfully applied to Group A *Streptococcus* and *Mycobacterium*.[28-30]

The hallmark of effective vaccine antigens is their ability to induce antibodies and/or to activate immune cells. Regarding this feature, in silico prediction of antigenicity is still in infancy. It is anticipated that with the wealth of knowledge currently being generated, it will be possible to develop prediction algorithms to pinpoint proteins likely to be immunogenic and/or protective.[31] More advanced is the strategy to mine genomic sequence databases of intracellular pathogens for predicted T-cell epitopes and validate them experimentally based on immune recognition.[32,33] Despite all successes, the bioinformatic genome mining approach has limitations due to the inaccuracy of available algorithms, regarding the prediction of (1) open-reading frames that encode proteins; (2) surface and secreted proteins; (3) gene function based on homology searches. Moreover, it is almost impossible to predict the conditions under which candidate antigens are expressed, unless the genes are equipped with well-defined regulatory sequences and promoters.

The availability of complete pathogen genome sequences stimulated the development and wide-spread application of high density DNA-arrays. Comparative microarray analysis identifies genomic diversity and conservation patterns among bacteria. The development of vaccines cross-protective among serotypes and variants of pathogenic species specifically profits from this

analysis, as it was demonstrated by the identification of common genes and protective antigens from major serotypes of *Streptococcus agalactiae*.[34,35]

Profiling of genomic expression with microarrays has revolutionalized the analysis of genes involved in microbial pathogenesis (reviewed in ref. 36). Considering its value in vaccine development, the emphasis is focused on pathogen-host interactions. In several studies novel vaccine candidates were identified, based on requirement for infectious state and dissemination, adhesion or evasion of innate defense mechanisms.[37-40] This approach—that heavily relies on genome annotation and bioinformatics—is most powerful in providing a global view on integrated cellular processes active during infection. Again, it has to be followed by combined application of gene cloning, recombinant protein technology and in vitro functional assays to validate target selection for vaccine development.

Proteome analysis has rapidly developed in the postgenome era and is now widely accepted as a complementary technology to genetic profiling (reviewed in ref. 41). The most direct way of using proteomics technologies for antigen identification is the combination of conventional proteome analysis with serology. There have been a number of recent studies investigating the "immunoproteome" of important human pathogens (for an example see Haas et al[42]). Combining "reverse genomics" and proteomics is especially useful for confirmation of bioinformatic prediction of ORFs and surface location. Moreover, a strong asset of proteomic studies is the identification of surface located proteins that cannot be predicted by bioinformatic means.[43,44] Serological proteome analysis of enriched membrane and cell wall fractions from several pathogens, such as *S. aureus*, *Bacillus anthracis* and *S. agalactiae* has indeed demonstrated to identify novel surface antigens and protective vaccine candidates without sequence features that could have been recognized by in silico prediction algorithms.[45-47]

The design of proteome-based studies has to be carefully performed, since there is an inherent risk to preferentially detect abundant proteins and to miss those that are expressed only under in vivo conditions and have lower solubility (e.g., membrane and surface proteins). Another need, not necessarily met by proteome analysis, is that protective vaccine components have to be derived from proteins expressed under disease conditions against which prevention is directed. As many virulence factors and antigens are only expressed in vivo, approaches that solely rely on in vitro grown bacteria are likely to miss important protective antigens.

Evaluation of immune responses against any candidate antigen is a crucial validation task and cannot be circumvented. Therefore, techniques using human immunogenicity as their primary screening and selecting parameter on a genome-wide basis seem to be especially valuable for vaccine development. Recently a novel approach combining the advantages of full genome coverage and serological antigen identification was published. The method was first applied to the genome-wide identification of in vivo expressed antigens from *S. aureus* by using antibodies from human serum and comprehensive small-fragment genomic surface display libraries.[22] Subsequently, the technology was extended with an integrated approach for antigen validation as selected clones are directly subjected to generation of epitope-specific immune sera for surface localization and in vitro functional assays. This feature allows the analysis of antigens without the demanding task of high through-put recombinant protein production. This method, named antigenome technology, has been extended to many important human pathogens and validated by the discovery of novel and highly protective antigens, in addition to the identification of the majority of the ones that have been previously described.[20] Since the antigenome technology provides a subset of all genome-encoded proteins, which are expressed by the pathogen in vivo and induce antibodies in humans, the identified antigens fulfill major requirements of vaccine candidate antigens. It is interesting to note that the antigens confined by the antigenome seem often to be involved, as secreted and surface bound proteins, in virulence functions and, thus, being attributed to the "pathosphere" that has been defined as the growing gene pool in which pathogens meet and mingle to cause diseases.[48] It is observed that many of the identified antigens from various pathogens were not or only very weakly expressed under in vitro growth conditions, indicating that a proteomic approach that preferentially selects abundant proteins would likely fail to identify them. As the bioinformatic genome mining approach depends on the accuracy

of available algorithms, potential vaccine candidates can be missed due to a misleading or not existing annotation. Based on the analysis of the antigenomes of fifteen pathogens, approximately 25% of all identified antigenic proteins can only be assigned to hypothetical proteins or proteins with unknown function. Many of the identified antigens would, thus, be not be found by a bioinformatic approach. The cumulative data obtained for the fifteen antigenomes showed that a large fraction of the antigens identified by this method represents cell surface or secreted proteins. Nearly fifty percent of all antigens fell into four cellular role categories: cell wall, cellular processes, transport and binding proteins and determinants of protein fate. In order to pinpoint candidates for vaccine development, a comprehensive and rapid validation strategy to retrieve the most promising antigens from the 100-200 antigens was implemented. Clones selected from peptide display libraries are directly subjected to generation of epitope-specific immune sera used for testing of surface localization and in in vitro bactericidal assays. The human immunogenicity of identified antigens is evaluated with synthetic peptide epitopes. The application of these major selection criteria combined with traditional gene conservation studies reduces the antigenome to a small number of candidate proteins that can be rapidly expressed in recombinant form for subsequent in vivo studies. The re-identification of most of the previously identified protective antigens of *Staphylococci* and *Streptococci*, such as PspA, M1 protein, Sip and ClfA gives further supports the power of the antigenome technology. Most importantly, novel protective proteins yielding animal protection in animal vaccine models, were found in the prioritized groups of antigens derived e.g., from *S. aureus*[23] and *Streptococcus pneumoniae* (unpublished data), respectively. Thus, the utilization of protective antigens—included in subunit vaccines—as targets for monoclonal antibodies, provides an attractive strategy to develop novel treatments against life threatening infections. Such a notion is supported by recent data showing that protection can be conferred to naïve animals, using serum directed against target antigens that have been validated in vaccine models (Nagy et al, personal communication).

The Advent of Monoclonal Antibodies in Disease Treatment

The renaissance of antibody therapy since the mid-1990s was mainly possible through significant improvements in antibody generation and purification (Fig. 1). The first step towards nowadays production technologies was the description of the unlimited generation of monoclonal antibodies by Georges Koehler and Cesar Milstein in 1975,[49] for which they were awarded the Nobel Prize in 1984. They fused mouse myeloma cells with normal antibody-producing splenic B-cells isolated from mice that were immunized with sheep red blood cells as antigen. The resulting hybridoma cells possessed the immortal propagation potential of the myeloma cells and secreted anti-sheep red blood cell antibodies. Selected clones could then be cultured indefinitely and secreted large quantities of monoclonal antibodies.

Despite their success as research tools, mouse monoclonal antibodies as human therapeutics are limited for various reasons. The main problem is the high immunogenicity of these foreign proteins in humans resulting in fast clearance (short half life) and toxicity by human anti-mouse antibodies (HAMAs).[50] Moreover, mouse antibodies have a reduced effect in human recipients due to their nonoptimal interactions with human complement and F_c receptors.[51]

In the early 1980s strategies for chimerization and humanization were ensued to overcome the limitations of mouse monoclonal antibodies. Chimerization demands the joining of the variable regions of mouse antibodies with the constant domains of human immunoglobulins that takes advantage of recombinant DNA techniques resulting in chimeric antibody derived from mouse and human antibody genes.[52] Although being less immunogenic than murine monoclonal antibodies, human antichimeric antibody responses have even been reported for chimeric antibodies.[53] To further reduce the undesirable immune response and confined inactivation, the mouse segment within the humanized monoclonal antibodies has been restricted to the complementarity determining regions (CDR) in CDR-grafted "humanized" antibodies.[54] In order to humanize a mouse monoclonal antibody, the closest matching human immunoglobulin allotype is first identified by structural comparison.[55,56] Then recombinant approaches are used to graft the CDRs from mouse hybridomas to the corresponding selected human immunoglobulin framework. As a result, the
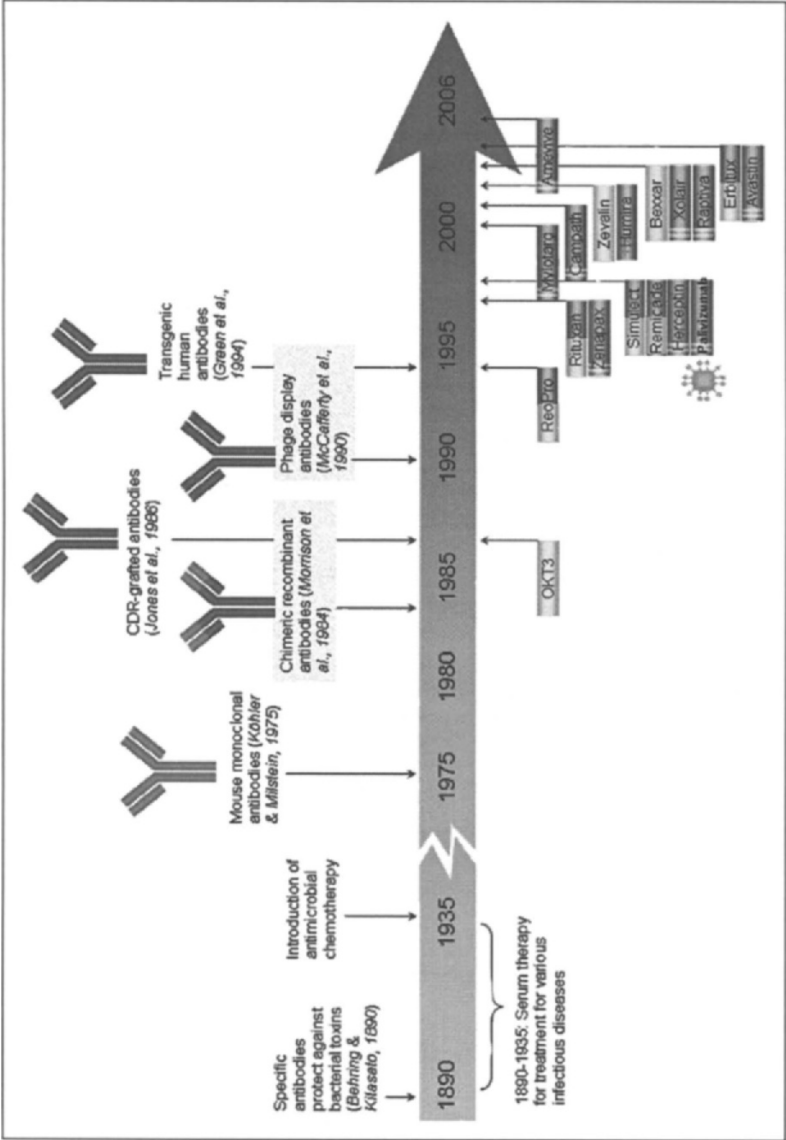
Figure 1. Evolution of antibody therapy and application in the clinic.[49,52,54,61,71] After extensive use of anti serum therapy at the beginning of the last century it was almost abandoned until the advent of monoclonal antibodies. In general new monoclonal antibodies were approved by the FDA 10 years after the development of a new technique was reported in the literature. Palivizumab is the only momodonal antibody currently used in the clinic that targets an infectious disease.

antibody only contains the antigen binding region from mouse origin, while the remainder of the variable and constant regions is derived from a human source.

While routine mouse monoclonal antibody production has been established, human monoclonal antibodies cannot be generated by conventional hybridoma technology, since it was not possible to found human cell lines that secrete constantly high levels of antibodies and, furthermore, humans cannot be challenged with all kind of antigens, due to ethical and safety reasons. Nowadays, phage display technology (reviewed in refs. 57-59) and transgenic mice with a human antibody locus (reviewed in ref. 60) represent established, widespread and robust technologies that allow the generation of potent human antibodies.

Phage display technologies enable in a simple to use and highly versatile procedure for the selection of antibodies against known or novel antigens. The phage display library (first description by McCafferty et al[61]) represents a collection of independent clones carrying a foreign DNA sequence encoding an antibody domain expressed as a fusion with the coat protein of mainly filamentous bacteriophages, as M13 or Fd (reviewed in ref. 62). Monoclonal antibody libraries can be recruited from immune fragments that are already biased towards certain specificities (encoded in the genome of immunized or infected animals or humans), or naïve unbiased fragments that can be derived from nonimmune natural or semi-synthetic sources, bypassing the need for previous immunization. By applying the best suitable selection procedures, those phages that bind to the target antigen with highest affinity are retained. The phages are enriched by selective adsorption to an immobilized antigen ("panning") (reviewed in ref. 12); however various specialized screening techniques exist.[57,63-65] Phage display provides the opportunity to mimic human immune response, also because of the high degree of natural variations found in the replication of the phage genomes.[66] B-cell maturation in vivo requires recombination of germline gene segments accompanied with changes and mutations that can be imitated in vitro by DNA random cloning of VH and VL chain genes.[67] The somatic hypermutation process that naturally contributes to the affinity maturation of antibodies can be achieved artificially by inserting point mutations into gene segments of complementarity determining regions.[68,69]

A method to circumvent the laborious steps of founding humanized and to obtain directly human monoclonal antibodies was developed by engineering transgenic mice with a human immunoglobulin locus as source for antibody producing hybridoma cell lines. (reviewed in ref. 60). Already in 1985 Alt et al proposed to exploit transgenic mice for the generation of therapeutic antibodies,[70] and as soon as 1994 the XenoMouse® (Abgenix, Inc.)[71] and the HuMAb Mouse® (Gen-Pharm-Medarex)[72] were reported to be the first mice carrying both the human VH and VL repertoire created via pronuclear microinjection or yeast protoplast fusion with embryonic stem cells, respectively. For monoclonal antibody generation B-cells are isolated from immunized mice and fused to hybridomas, in a similar manner to the traditional mouse monoclonal antibody production. By employing microcell-mediated chromosome transfer—a technique capable to transfer very large fractions of the human germline—Tomizuka et al generated a chimeric mouse—TransChromo Mouse™ (TC Mouse™) carrying human chromosomes 2 and 14 regions containing the human κ-light-chain and heavy-chain loci.[73,74] In order to increase the low efficiency of hybridoma production due to instability of the Igκ locus, the KM Mouse™ was created by cross-breeding the Kirin TC Mouse™ with the Medarex YAC-transgenic mouse.[75] These mice possess the capability to carry out VDJ recombination, heavy-chain class switching and even somatic hypermutation of human antibody genes in a normal mode to generate high-affinity antibodies with completely human sequences.[76] The resulting antibodies exhibit a half-life similar to natural human antibodies[77] and show only differences in glycosylation patterns, thereby representing a major improvement in hybridoma technology.[12] Although human monoclonal antibodies derived from transgenic mice have not yet paved their way up to FDA approval and registration, so far clinical trials with them have not revealed adverse immunogenic side events in patients,[78-80] in contrast to chimeric, CDR grafted or phage display derived monoclonal antibodies.[81] However there is still a need for confirming these promising data by testing transgenic mouse derived antibodies in larger subject cohorts.

On the other side, the success of phage display technologies in mimicking the in vivo antibody selection process in essence has led to intensive exploration of possible improvements, mainly in the field of new display techniques. All these new selection platforms share four major steps: (1) the creation of genotypic diversity; (2) the linkage between genotype and phenotype; (3) the application of a screening procedure; and (4) the amplification of the selected binding sites.

In the Ribosome and mRNA display method,[82] the antibody and its encoding mRNA are linked by the ribosome which is made to stop without releasing the polypeptide.[83-85] The use of e.g., nonproof-reading polymerases provides additional diversity between generations and therefore represents a very successful technique in the field of antibody affinity maturation.[86]

The attempt in displaying antibodies on the surface of different microbes has only been successful so far, when employing the yeast *Saccharomyces cerevisiae*.[87] Antibodies are displayed via fusion to the α-agglutinin yeast adhesion receptor on the cell wall and selection can be accomplished via flow cytometric cell sorting. Besides yeast display, a lately described *Escherichia coli* based approach is currently under development.[88]

Recently developed antibody platform technologies include retroviral display,[89] protein-DNA display,[90] microbead display by in vitro compartmentalization,[91] in vivo growth selection based on protein fragment complementation[92] and other techniques.[93] However, their advantages over more established systems remain to be demonstrated.

One problem in the application of monoclonal antibodies lies in their restriction to a single specific epitope, limiting their ability in eliminating dynamic and evolving targets and retaining activity in the event of antigen mutation. A new generation of therapeutic antibodies that may overcome the restriction of monoclonal antibodies is the development of a recombinant poly- or oligoclonal antibody technology.[94] For the generation of "Symphobodies"—fully human, antigen-specific recombinant polyclonal antibodies—antibody producing cells are isolated from naturally immune donor blood. cDNA encoding human heavy and light chains are amplified and linked together by Symplex PCR™; pooled PCR products are then inserted into an expression vector and screened for antigen binding. Constructs expressing the selected antibodies are cloned into Chinese hamster ovary cells where they are site-specific integrated into the genome.[94] Thus, such a development of a human antibody repertoire mirrors the human polyclonal immune response against specific antigens.

Besides the fact that the recombinant expression of antibody genes is often difficult because of their large size, the usage of whole immunoglobulins sometimes causes undesired side effects that are mediated by the Fc part of the antibody. To overcome such problems antibody fragments such as Fab, scFv, diabodies and minibodies have been engineered by removing either the entire constant region or the Fc portion.[12] Advantages shared by these antibody fragments include their better clearance from whole body, better tissue/tumor penetration characteristics and their simple and straightforward production in bacteria bypassing mammalian cell based production. The smallest fragments are single chain fragment variables (scFv) formed by tandem arrangement of the VH and VL domains joined by a flexible linker peptide exhibiting a comparable affinity of a Fab.[95,96] Their biological effects can be enhanced through linker length reduction that generates noncovalent scFv dimers "diabodies";[97] by further shortening trimers[98] or even tetramers can be formed.[99] ScFvs have also been modified to deliver toxins and chemotherapeutics to various tumors by binding to cancer-associated antigens, e.g., by coupling the *Pseudomonas aeruginosa* exotoxin A to scFv.[100] Linking of scFvs of different specificity creates bispecific antibodies that bind two different structures on single or different cells.[101] Other truncated antibody variants are Minibodies—homodimers of scFv-CH3 fusion proteins—and Flex minibodies—scFv-IgG1 hinge region fusion proteins.[102]

Whole antibody molecules can be modified as well by coupling with anti-microbial drugs. Antibodies possessing specificity to microbial antigens can be simultaneously linked to toxins, acting as immunotoxins that way. For example, Human Immunodeficiency Virus (HIV) and Cytomegalovirus specific antibodies have been linked to the ricin A chain or the *Pseudomonas* Exotoxin A.[103-105] Unfortunately toxins can elicit immune responses limiting their repeated

therapeutic use. An alternative represents the linking of radionuclides to specific antibodies that do not need to be internalized, like toxins and are unlikely to produce significant immune responses. Radionuclide-labeled antibodies have been tested against *Cryptococcus neoformans* and pneumococcal infections in mice.[106,107] Another development in modifying the antibody molecule was the creation of bispecific antibodies carrying two different Fab fragments and recognizing a microbial epitope for pathogen binding and at the same time a host immune component. This strategy was shown to be successful in animal models for the clearance of bacteriophages[108] and *P. aeruguinosa*.[109]

The application of humanized and even fully human antibodies—is associated with low toxicity and high specificity. The benefit of high specificity is that only disease-causing pathogens are targeted and therefore the host flora should not be altered or resistant microorganisms be selected. A caveat is that pathogens with high antigenic variation may require more than one monoclonal antibody for therapy and mutants lacking the antibody determinant could emerge during treatment. Antibody molecules are highly versatile; by binding to a single determinant they can mediate various biological effects including toxin neutralization, microbial opsonization, complement activation and antibody-directed cellular cytotoxicity (Fig. 2). Antibodies can also be used to target host cells and enhance immune functions especially desirable against infectious diseases and tumors or to suppress immune responses by reducing the number of immune cells, neutralizing cytokines or blocking receptors.
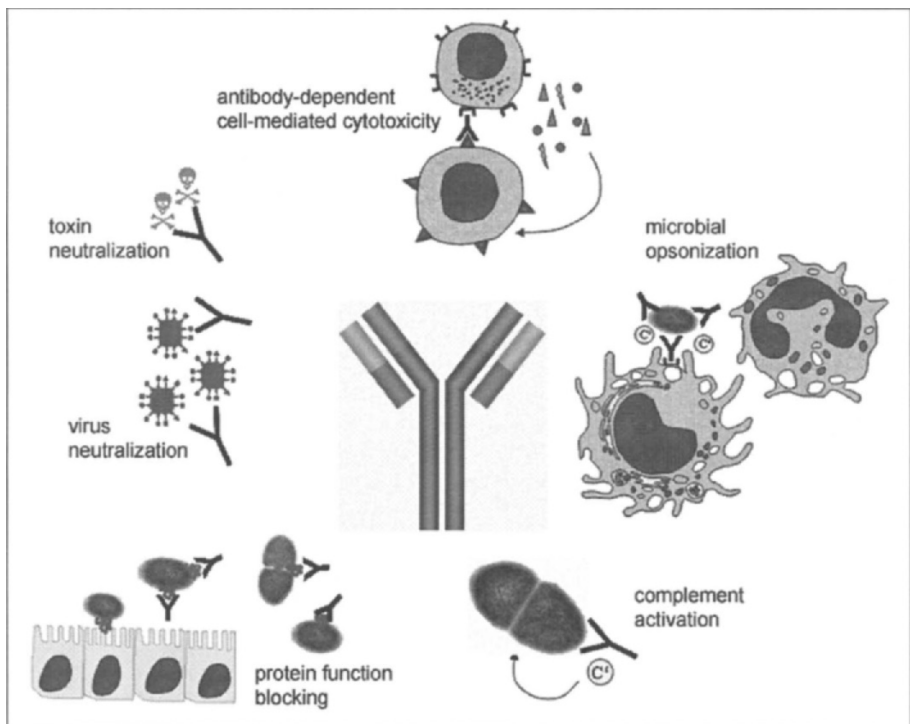

Figure 2. Biological effects of antibodies in infectious disease. Antibodies neutralize viruses and toxins, block protein functions important for microbial adherence or growth, activate complement and microbial opsonization and are a prerequisite for cell-mediated cytotoxicity. All these functions together facilitate the host to combat the invading pathogen (Adapted from Casadevall et al.[8])

The major disadvantages of antibody based therapies are high costs associated with production, storage and administration. Since antibodies have to be produced in live expression systems, the risk of contamination with prions or viruses requires continuous monitoring and testing. Additionally, antibodies have to be administered shortly after infection to be efficient, requiring rapid microbiological diagnosis. Additionally manufacturing of Symphobodies, mimicking polyclonal antibodies in human immune response, may still have to prove that they can be obtained without chance in their composition under stable GMP conditions.

From Serum Treatment to Anti-Infective Monoclonal Antibodies

In the late 19th century Behring and Kisato discovered the efficacy of immune sera in treating infectious diseases, such as diphtheria and tetanus.[110] In 1891 the Klemperers already protected rabbits against *S. pneumoniae* with immune sera showing the potential usefulness of passively administered antibodies for the treatment of pneumococcal infections.[111] However reliable anti-pneumococcal therapy was not available until the mid 1920s, since the development of successful serum therapy required the discovery that pneumococci are genetically diverse and only type-specific sera provide protection. Improved vaccination schedules for serum donors to generate good immune responses and advanced antibody purification techniques, as well as the standardization of serum potency were necessary steps in the introduction of serum therapy (reviewed in ref. 112). The high death rate associated with meningococcal meningitis lead to fast developments also in this sector; a significant reduction of the case fatality rates was already achieved with horse sera in the early 20th century.[112,113]

Serum therapy reached its heyday in the 1920s to the mid 1930s when it was standard clinical practice in the treatment of a variety of infectious diseases caused by *S. pneumoniae, C. diphteriae, Neisseria meningitidis, Haemophilus influenzae, Streptococcus pyogenes* and *Clostridium tetani*. The broad application of serum as treatment for pneumococcal disease can be estimated regarding advertisements of that time in medical journals (Fig. 3). However, with the advent of anti-microbial chemotherapy passive immunization with serum was largely abandoned for the treatment of bacterial infections due to major advantages in being less toxic, more effective and cheaper. Serum therapy was often associated with severe side effects including fever, chills and allergic reactions and delayed toxicity called "serum sickness" a syndrome associated with rash, proteinuria and arthralgia. Moreover, for satisfying efficacy precise diagnosis, appropriate and nondelayed dosage was necessary asking for physicians with considerable experience. The production of horse or rabbit therapeutic sera was very expensive because of the need for animal facilities, purification techniques, adequate storage and standardization. Nevertheless lot-to-lot variation could not be fully eliminated (reviewed in ref. 114).

Upon the arrival of the antibiotic era, anti-sera were still used for toxin-mediated disease such as botulism, tetanus and diphtheria in addition to anti-toxin therapy in the treatment of venomous snake bites.[11] The lack of efficient anti-viral treatments also stimulated the use of antibody preparations as postexposure prophylaxis in e.g., rabies or hepatitis B (reviewed in ref. 9).

In spite of the previously experienced shortcomings, long-time neglected antibody based therapies face a renaissance today. The description of hybridoma technology in 1975[49] fired researcher's imagination in developing new therapies against cancer, autoimmune or infectious diseases. As early as at the dawn of the 20th century Paul Ehrlich already dreamed about the use of antibodies as "magic bullet" for the treatment of cancer. Indeed, in the mid' 1980s the first efficient use of a monoclonal antibody for the treatment of refractory lymphoma was reported.[115] The anti-tumor effect was only temporary, since murine monoclonal antibodies have only short in vivo half life and are immunogenic in humans; moreover they don't kill target cells forcefully due to low efficiency in complement activation and antibody dependent cell cytotoxicity. The first FDA approved murine monoclonal antibody for clinical use was OKT3 targeting CD3 in 1986 and was designed for prevention and treatment of organ rejection.[116]

Fortunately, monoclonal antibody techniques underwent continuous and tremendous improvements in reducing the mouse derived portion of the protein and enabling the production of

Figure 3. Advertisement for serum therapy for Type I *Pneumococcus*. Lederle (now Wyeth) successfully sold pneumococcal treatments already in the early 20th century.

chimeric, humanized and nowadays even fully human antibodies. In the 30 years since the invention of hybridoma technique 21 monoclonal antibody therapies have been approved by the FDA (source: AdisInsight, 07.07.2006); only a single one targets an infectious disease—Palivizumab (Synagis®) against respiratory syncytial virus infections.

In spite of the incredible efforts undertaken to develop novel antibody based treatments with hundreds of monoclonal antibodies being currently under preclinical development or clinical testing, only the minority of these efforts are directed against infectious targets. Among viral infections, AIDS is far the most explored area (Table 2). Due to the extreme variability of neutralizing HIV epitopes, in addition to those combating the virus particle itself,[117,118] many monoclonal antibody approaches target host molecules (such as CTLA-4, CD4, LFA-1, CCR5) to hinder viral entry (reviewed ref. 119). Emerging viral infections caused by the SARS corona virus and West Nile Virus also attracted the attention of monoclonal antibody developers and several preclinical efforts are expected to enter clinical development (Table 2).

Due to the widespread appearance of multi-drug resistant bacterial pathogens and the increasing population of immuno-compromised patients, more and more efforts are focused on antibody-based strategies against pathogenic bacteria and fungi. Especially considerable efforts were and are still undertaken to treat septic shock caused by gram-negative bacteria via neutralization of endotoxin and of TNF-α induced early in the disease, unfortunately with no successful outcome so far.[120-123] The most frequent microbial targets of new developments are opportunistic nosocomial pathogens, such as *S. aureus* and *epidermidis*, *P. aeruginosa* and Candida species (Table 1 and Table 3). The molecular targets for these monoclonal antibodies are surface structures of these pathogens, including capsular polysaccharides, cell wall glycolipids and surface proteins. The primary aim is to increase opsonophagocytic elimination of the respective organisms with the help of the host's immune cells. Unfortunately, in immuno-compromised patients (under anti-tumor treatment, organ transplantation, old age), the number of effective phagocytic cells is significantly lower than in healthy people and relying only on opsonophagpcytosis may not be sufficient for cure. Monoclonal antibodies that target surface proteins and that also have essential functions in in vivo survival, multiplication (cell division, nutrient acquisition) and pathomechanisms (adhesion, cytotoxicicity, immune evasion), offer another opportunity to reduce bacterial growth and ameliorate infectious damage to the host.[124-127] A single chain anti-fungal antibody that was selected by the hsp90 protein of *Candida albicans* from antibody cDNA libraries of patients who recovered from invasive candidiasis is being developed (Mycograb®). It consists of the antigen-binding variable domains of antibody heavy and light chains linked together to a recombinant protein that is expressed in *E. coli*. Mycograb® is not dependent on recruitment of white blood cells or complement, but simply acts by binding and inhibiting hsp90 of Candida.[128]

Current fear of bioterrorism using biological weapons encourages the development of antibody therapies against anthrax, botulism, ebola or smallpox virus infections and aims to provide immediate immune protection through antibodies that either neutralize the pathogens and toxins themselves, or target the host by blocking corresponding receptors to prevent infection or toxicity. Recently Cohen and coworkers demonstrated the inhibition of the lethal effect of anthrax toxin via blocking of its human coreceptor, LRP6 with LRP6 specific antibodies.[129]

The Next Chapter of the Antibody Success Story: Bacterial Infections

In spite of the historical landmark therapy against diphtheria, antibody therapy against bacterial infections, only exceptionally, has entered the medical arena in the last 70 years. The advent of antibiotics during the forties has certainly discouraged the development of further serum treatments against bacterial pathogens.

Antibiotics have seemingly become a relatively cheep and mostly reliable weapon to control most bacterial infections and epidemics. Alongside with the increase of hygienic standards, the penetrations of mandatory childhood vaccinations and antibiotic treatment, bacterial infections seemed to be a medical problem confined only to less developed parts of the world. The cost-efficient availability, the seemingly ever growing pipeline of novel antibiotics with increasing efficacy

Table 2. Therapeutic antibodies against viral infections

Drug	Pathogen	Antigen Target	Type of Antibody	Highest Phase	Originator	Indication
Palivizumab	RSV	RSV F glycoprotein	Humanised mAb IgG1	Launched	MedImmune	RSV infections
Motavizumab	RSV	RSV F glycoprotein (derived from Palivizumab)	Humanised mAb IgG1	III	Eli Lilly/ MedImmune	RSV infections
2G12, 2F5, 4E10	HIV	HIV gp120, HIV gp41	Human mAb	II	Polymun Scientific	HIV infections treatment & prevention
hNM01	HIV	V(3) region of the HIV-1 envelope protein gp120	Humanised mAb	I	SRD Pharmaceuticals	HIV infections treatment
HIV gp41 mAb	HIV	HIV gp41 surface glycoprotein	Fully human mAb	Preclinical	Medarex/Pfizer	HIV infections treatment
HEBIG™	Hepatitis B	Undisclosed	Pooled plasma of individuals with high titers of antibody to the hepatitis B surface antigen	Launched	Nabi Biopharmaceuticals	Hepatitis B
Civacir™	Hepatitis C	Undisclosed	Plasma of infected patients with high levels of anti-hepatitis C virus (HCV) antibodies	I	Nabi Biopharmaceuticals, Novartis	Hepatitis C
XTL 6865	Hepatitis C	HCV envelope E2 protein	Fully human (derived from human immune cells of convalescent patients)	I	XTL Biopharmaceuticals	Hepatitis C
Anti-SARS mAb	Severe acute respiratory syndrome (SARS)	SARS coronavirus glycoprotein S	Fully human mAb	Preclinical	Medarex/ Massachusetts Biologic Laboratories	Coronavirus infections

Table 2. Continued

Drug	Pathogen	Antigen Target	Type of Antibody	Highest Phase	Originator	Indication
Anti-SARS mAb	SARS	SARS coronavirus glycoprotein S	Fully human mAb	Preclinical	Crucell	Protection against SARS virus infection
Anti-M2 mAb	Influenza virus	Influenza A M2 protein	Fully human mAb	Preclinical	Kirin/Corixa Corporation	Influenza virus infections
WNV mAbs	West Nile Virus (WNV)	Domain II and III of WNV envelope protein	Fully human mAb	Preclinical	Crucell	WNV infections prevention and treatment
Anti-rabies mAbs	Rabies	Rabies virus glycoprotein	Fully human mAb	Preclinical	Crucell/CDC	Rabies
Anti-hMPV mAb	Human metapneumo-virus (hMPV)	Undisclosed	Undisclosed	Preclinical	MedImmune	Metapneumo-virus-infections
Ipilimumab	HIV	CTLA-4	Fully human mAbs	I	Medarex	HIV infections treatment, among other applications
TNX 355	HIV	CD4 receptor	Humanized mAb 5A8	II	Biogen Idec	HIV infections treatment
Cytolin®	HIV	4 epitopes of the alpha sub-unit of LFA-1 (CD8)	Murine mAb	II	CytoDyn	HIV infections treatment
PRO 140	HIV	Human CCR5	Humanised mAb	I	Progenics Pharmaceuticals	HIV infections treatment

Table 2. *Continued*

Drug	Pathogen	Antigen Target	Type of Antibody	Highest Phase	Originator	Indication
CCR5mAb004	HIV	Human CCR5	Fully human	I	Human Genome Sciences	HIV infections treatment
mAb 1F7	HIV	Idiotype common to anti-HIV and anti-SIV antibodies	Murine mAb IgMκ	Preclinical	Immune Network	HIV infections treatment
mAb B4	HIV	HIV receptor complex	Murine mAb	Preclinical	United Biomedical	HIV postexposure prophylaxis
mAb A3D8	HIV	CD44	Murine mAb	Preclinical	Duke University Medical Center	HIV-1 infections, myeloid leukaemia
CFY 196	Rhinovirus	ICAM-1(receptor for HRV)	Tetravalent humanised Fab fusion protein	Preclinical	Perlan Therapeutics	Rhinovirus infections
Anti-IFN-γ polyclonal antibody	Herples Simplex virus (HSV)	Interferon-γ (IFN-γ)	Polyclonal antibody	I	Advanced Biotherapy	HSV infections treament, among other applications
Bavituximab	Viruses	Phospholipid abnormally exposed on vascular endothelium of tumor blood vessels	Chimeric antibody	I	Peregrine Pharmaceuticals	Cancer, Hepatitis C treatment, Influenza virus infections, Viral infections

Data were collected from the Adis R&D Insight database and homepages of listed companies in July 2006 using the world wide web. Data were grouped into: first, antibodies targeting the virus particle itself and then second, those targeting host structures. Monoclonal Antibodies are abbreviated with mAbs. This is not a complete list.

Table 3. **Antibody based therapies against fungal infections**

Drug	Pathogen	Antigen Target	Type of Antibody	Highest Phase	Originator	Indication
Mycograb®	*Candida spp.* (*C. albicans, C. krusei, C. parapsilosis, C. tropicalis*)	Heat shock protein 90 (hsp90)	Human genetically recombinant antibody	Preregistration	NeuTec Pharma	Treatment of systemic candidiasis, Cancer (e.g., Breast cancer)
Candida MAb	*Candida spp.*	MSCRAMM® proteins	Undisclosed	Preclinical	Inhibitex	Treatment of candidiasis
ACE 5033	*Aspergillus fumigatus*	*A. fumigatus* surface protein	Fully human mAb	Preclinical	ACE BioSciences, Genmab	Aspergillosis, Mycoses
Candida mAb	*C. albicans*	Corixa antigens	Fully human mAb	Preclinical	GlaxoSmithKline and Amgen	Fungal sepsis caused by *C. albicans*

Data were collected from the Adis R&D Insight database and homepages of listed companies in July 2006 using the world wide web. Monoclonal Antibodies are abbreviated with mAbs. This is not a complete list.

invading the market has established the attitude in the medical community up into the 1970s of the last century that bacterial diseases may belong to the past. However, the emerging pattern of multidrug-resistant strains of an increasing number of pathogens in hospitals and communities has quickly ended the optimistic belief that the repertoire of anti-bacterial treatments will suffice the challenges in the infectious disease arena (for review see ref. 130). Also the discovery, development and registration of novel antibiotics have not fulfilled the too optimistic expectations that new registrations of treatments may bounce off the threat of untreatable bacterial infections (see commentaries by Clarke[131] and in Biocentury[132]). The infiltration of genomics,[133] intelligent drug design and molecular studies of bacterial host interactions in antibiotic development[134] has rather led to the sobering recognition that the number of suitable targets for new anti-bacterial drugs may be rather limited.[135-137] Furthermore, the often severe side affects, including allergic reactions against specific antibiotics, is restricting their applications, sometimes in critical medical conditions when they are most needed. Last, not least antibiotics often lead to lysis of bacterial cells and thereby freeing endotoxins at high levels, thereby causing overshooting immunity including sepsis.[138]

On the other hand before the advent of the monoclonal antibody technology, treatments of bacterial diseases with antibodies have been out of the reach of economical feasibility. Production of antibodies by immunizing animals as resorts to obtain serum is not a trivial process regarding quality, reproducibility and unwanted contaminations. Also as one has experienced with whole cell bacterial vaccines, immunization with in vitro grown pathogens may not lead to the type of specific antibodies that neutralize them, since they may not display the proper antigens at the surface. Thus, the progress made in defining disease specific antigens for vaccine development has provided novel tools to raise highly specific antibodies that may prevent or block bacterial infections or at least supporting the recovery process.

The skepticism in the medical and scientific community towards the paradigm of anti-infective anti-bacterial monoclonal antibodies is nurtured by multiple lines of thoughts:

1. Existing treatments are sufficient to control bacterial diseases.
2. Monoclonal antibody therapy may not find its way into treatment schedules that would justify the costs.
3. A single monoclonal antibody directed against a specific antigen per se may not be able to counteract the pathogenic course of a bacterial infection.

While all three arguments are widely accepted, a closer look into the paradigm discloses that they are not necessarily substantiated, if one considers the medical need, the progress made in identification of suitable antigenic targets and the positive experience of using monoclonal antibodies against e.g., malignant diseases (reviewed in ref. 139).

The medical need is given, whenever conventional treatment and prophylaxis are not available. *S. aureus* in context with nosocomial infections is equally a target for antibody treatments as *Pneumococcus*, both representing problem germs in intensive care (Table 4).

Moreover, costs for antibody treatments in connection with above described infectious disease outbreaks often missing adequate medical treatments appear to be not too dramatic, if one relates them to the hospital conditions and the underpinning economical efforts spent. Last but not least, the increasingly wide usage of monoclonal antibodies outside of the infections disease area has certainly aided in lowering the costs of development and manufacturing, thereby paving the way to novel treatments.[8]

The question remains what kind of features form the prerequisites for a monoclonal antibody in order to be able to counteract a bacterial infection? The answer to this problem lies—to our opinion—in the selection of the best suitable antigenic targets for the development of monoclonal antibodies. The antigens should be expressed on the pathogen surface during the infectious process; preferred throughout the most important stages of disease manifestation: i.e., during colonization, spreading and invasion. Also the antigens of choice should have a proven record to be a target of antibodies from individuals who have encountered the pathogen with positive or protective outcome. In addition, the selected antigens should be conserved among all clinical strains of the germ causing the underpinning infections.

Table 4. Medical need for novel therapeutics against nosocomial pathogens

Pathogen	Disease	Target Group	Incidence	Medial Need
Nosocomial	Sepsis, bacteremia, pneumonia, wound and surgical site infection, osteomyelitis, urinary tract infections	Hospitalized (esp. elderly, immunocompromized)	Nosocomial infections: 2 million/yr/US; 1 of 20 hospital admissions; 100.000 death/yr/US	High incidence High medical costs (20 Bn$/yr in dev. World) Increasing antibiotic resistance
S. aureus	Sepsis, bacteremia	Premature newborns, <34th pregnancy week	1-2/1000 life birth	Support the premature immune system
S. aureus	Sepsis, bacteremia, pneumonia, wound infection, osteomyelitis	Hospitalized, esp. surgery patients	25% of nosocomial infections	High mortality High incidence High medical costs Multi-drug resistance
S. pneumoniae/ Pneumococcus	Bacteremia Pneumonia Meningitis	Community acquired, elderly	~75.000/yr/US ~500.000/yr/US ~5.000/yr/US	High mortality High incidence High medical costs Antibiotic resistance
Group B Strep/ S. agalactiae	Bacteremia, pneumonia, meningitis	Premature newborns, <34th pregnancy week	1-2/1000 life birth	Support the premature immune system, increasing ab resistance
P. aeruginosa S. aureus	wound infection, bacteremia, sepsis	Intensive care, burn patients	80% of 2nd and 3rd grade burn patients	High mortality High medical costs Multi-drug resistance
P. aeruginosa	Pneumonia, sepsis	Intensive care, artificially ventilated	15-20% of HAP and 80% of VAP	Multi-drug resistance, high mortality, high medical costs
E. faecalis and faecium	Sepsis, bacteremia, endocarditis	Abdominal surgery patients	10% of nosocomial infections	Mortality, multi-drug resistance (E. faecium)
Klebsiella pneumoniae	Nosocomial pneumonia	Hospitalized (esp. elderly, immunocompromized)	10% of nosocomial infections	Multi-drug resistance

Hospital associated pneumonia (HAP) makes up 5-10/1000 hospital admissions and ventilation associated pneumonia (VAP) accounts for 80% of all HAP. Hospitalized burn patients average out 50.000 per year in the US. Of all approximately 9 million life births in the US and Europe per year premature birth before the 36th week accounts for 25% and premature birth before the 34th week for 10%.

All up to here listed features of target antigens may suffice the need to detect the intruder with a monoclonal antibody, particularly if the bound antibody funnels the bacterium into the immunological decontamination program, e.g., opsonization. On the other hand, the lesson learnt from antibiotics is that they have to kill the pathogen or at least disable bacterial growth in the host. In the light of the notion that prevention or treatment of a bacterial infection with monoclonal antibodies may be restricted to a single antibody, one would aim the target antigen also to exert a function needed for bacterial survival in the host. Thus, the antibody will neutralize a virulence factor or an enzyme needed in the infections life cycle of the pathogen. Such a dual mode of action resembles the features of monoclonal antibodies employed in cancers therapy: these antibodies seem to block cancerous cells by marking them for the immunological destruction, but also by blocking their growth. Thus, monoclonal antibodies need to be directed against carefully selected antigenic targets in order to achieve an optimum of interference with bacterial survival in the host.

The recently invented antigen identification procedure that is designed to establish the "antigenome" of pathogens has been instrumental in the development of novel bacterial subunit vaccines.[20-22] Characterization of a *S. aureus* antigen derived from the antigenome—that is presently used in preclinical and clinical programs—has indeed revealed its involvement in virulence and survival function.[23,128,140]

The feasibility of antigens to serve as targets for monoclonal antibody treatments can be pretested in vaccine models where protection of pathogen-challenged animals is accessed.[106,107,109,124,129,141-152] There is no doubt in mind that antigens giving the wanted protection in a vaccine model may not be sufficient when employed for the development of anti-infective antibodies. However, the potency of an antigen in providing protective immunity as vaccine may be a positive and sufficient selective criterion, alongside with all the other features that have been described for antigens selected for subunit vaccine development.

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