THERAPEUTIC ANTIBODIES FOR HUMAN DISEASES AT THE DAWN OF THE TWENTY-FIRST CENTURY

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Antibodies are highly specific, naturally evolved molecules that recognize and eliminate pathogenic and disease antigens. The past 30 years of antibody research have hinted at the promise of new versatile therapeutic agents to fight cancer, autoimmune diseases and infection. Technology development and the testing of new generations of antibody reagents have altered our view of how they might be used for prophylactic and therapeutic purposes. The therapeutic antibodies of today are genetically engineered molecules that are designed to ensure high specificity and functionality. Some antibodies are loaded with toxic modules, whereas others are designed to function naturally, depending on the therapeutic application. In this review, we discuss various aspects of antibodies that are relevant to their use as as therapeutic agents.

IMMUNOGLOBULIN DOMAIN
Compactly folded globular units of approximately 110 amino acids that comprise immunoglobulin heavy and light chains.

CYTOKINES
A class of small proteins released by one cell that affects the physiology of other cells locally and systemically in a particular fashion through binding to a specific receptor.

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Antibody structure

The typical antibody — or immunoglobulin (Ig) — consists of two antigen-binding fragments (Fabs), which are linked via a flexible region (the hinge) to a constant (Fc) region (FIG. 1). This structure comprises two pairs of polypeptide chains, each pair containing a heavy and a light chain of different sizes. Both heavy and light chains are folded into IMMUNOGLOBULIN DOMAINS. The 'variable domains' in the amino-terminal part of the molecule are the domains that recognize and bind antigens; the rest of the molecule is composed of 'constant domains' that only vary between Ig classes (BOX 1). The Fc portion of the Ig serves to bind various effector molecules of the immune system, as well as molecules that determine the biodistribution of the antibody.

Mechanisms of *in vivo* action

In antibody-based therapies, the goal is to eliminate or neutralize the pathogenic infection or the disease target, for example, bacterial, viral or tumour targets. Therapeutic antibodies can function by three principal modes of action: by blocking the action of specific molecules, by targeting specific cells or by functioning as signalling molecules. The blocking activity of therapeutic antibodies is achieved by preventing growth factors, CYTOKINES or other soluble mediators reaching their target receptors, which can be accomplished either by the antibody binding to the factor itself or to its receptor. Targeting involves directing antibodies towards specific populations of cells and is a versatile approach; antibodies can be engineered to carry effector moieties, such as enzymes, toxins, radionuclides, cytokines or even DNA molecules, to the target cells, where the attached moiety can then exert its effect (for example, toxins or radionuclides can eliminate target cancer cells). The natural EFFECTOR FUNCTIONS of antibodies are associated with binding to Fc receptors or binding to complement proteins and inducing COMPLEMENT-DEPENDENT CYTOTOXICITY (CDC). Targeting antibodies can retain such effector functions intact or they can be abolished during the design of the antibody, depending on the therapeutic strategy. The signalling effect of antibodies is predicated on either inducing crosslinking of receptors that are, in turn, connected to mediators of cell division or PROGRAMMED CELL DEATH, or directing them towards specific receptors to act as agonists for the

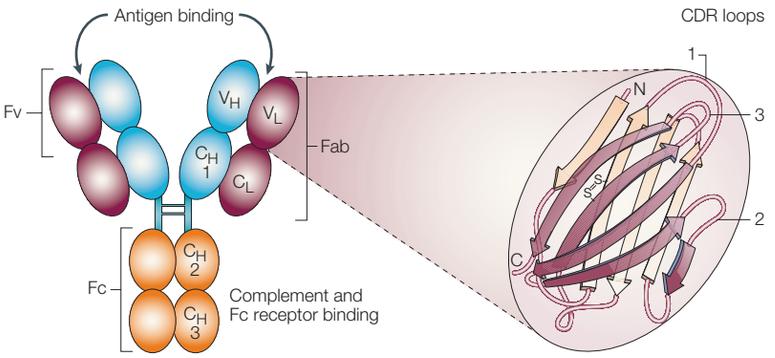

Figure 1 | **The modular structure of immunoglobulins.** This figure shows a single immunglobulin (Ig) molecule. All immunoglobulin monomers are composed of two identical light (L) chains and two identical heavy (H) chains. Light chains are composed of one constant domain (C_L) and one variable domain (V_L), whereas heavy chains are composed of three constant domains (C_H1, C_H2 and C_H3) and one variable domain (V_H). The heavy chains are covalently linked in the hinge region and the light chains are covalently linked to the heavy chain. The variable domains of both the heavy and light chains compose the antigen-binding part of the molecule, termed Fv. Within the variable domains there are three loops designated complementarity-determining regions (CDRs) 1, 2 and 3, which confer the highest diversity and define the specificity of antibody binding. The Fc portion is glycosylated and contains the sites for interaction with effector molecules, such as the C1 complex of the complement system and a variety of Fc receptors including the neonatal Fc receptor (FcRn).

activation of specific cell populations. Another approach is to use antibodies as delivery vehicles for DNA and, more recently, to deliver antigens to certain immune cells that present processed antigenic peptides, or epitopes, to T cells, to activate a specific immune response against that antigen.

The development of therapeutic antibodies

Target specificity in the treatment and prophylaxis of diseases such as infection, cancer and autoimmune disorders has become more viable through the development of monoclonal antibodies. The mouse HYBRIDOMA technology described by Köhler and Milstein was an important step in the development of antibody technology and paved the way for the emergence of therapeutic monoclonal antibodies[1]. During the 1980s, resources were directed towards the evaluation of the *in vivo* use of mouse monoclonal antibodies in humans, aimed at both imaging and therapy[2]. Mouse monoclonal antibodies were shown to have limited use as therapeutic agents because of a short serum half-life, an inability to trigger human effector functions and the production of human antimouse-antibodies[3] (the HAMA response). In an attempt to reduce the immunogenicity of mouse antibodies, genetic engineering was used to generate chimeric antibodies, that is, antibodies with human constant regions and mouse variable regions[4,5]. However, although chimeric antibodies were perceived as less foreign, and therefore less immunogenic, than mouse monoclonal antibodies, human anti-chimeric antibody responses (HACAs) have nonetheless been observed[6]. Further minimization of the mouse component of antibodies was achieved through CDR (complementarity-determining region) grafting[7] (FIG. 2). In such 'humanized' antibodies, only the CDR loops that are responsible for antigen binding are inserted into the human variable-domain framework. The ability to manipulate antibodies into more human variants finally made antibodies useful for clinical use. With the isolation of genes encoding human variable regions, their successful expression in *Escherichia coli*[8,9] and the introduction of phage-display technology[10], the task of selecting fully human variable domains has been greatly simplified.

Selection from phage-display libraries of human antibody fragments is today the most used and well-established technology for the development of new human antibodies (FIG. 3a). Another approach is to use mice that are transgenic for the human Ig locus[11]. Immunization of such a transgenic mouse results in a human antibody response, from which hybridomas that produce human antibodies can be generated (FIG. 3b).

Today there are approximately 200 antibodies in clinical trials and the US Food and Drug Administration has approved several antibodies against cancer[12,13], transplant rejection[14], rheumatoid arthritis and Crohn's disease[15,16], and antiviral prophylaxis[17] (TABLE 1). So far, 20% of all biopharmaceuticals in clinical trials are monoclonal antibodies (examples are shown in TABLE 2, and see clinical trials web site in online links box), making this the second largest biopharmaceutical product category after vaccines. As the development of potential new therapeutic agents into commercial products takes about 10 years, the FDA-approved antibodies, and some of those in the end-stages of development pipelines, are chimeric or humanized antibodies that were developed with early antibody engineering technologies. The more recently developed reagents, on the other hand, are completely human antibodies that are derived from phage antibody libraries and transgenic mice[17–20].

Antibodies against infectious agents

In the pre-antibiotic era, SERUM THERAPY was widely used as treatment for infectious diseases such as anthrax, smallpox, meningitis and the plague. With the introduction of vaccines and antibiotics, the use of serum therapy declined. However, with the reduction and eradication of certain diseases (for example, smallpox), which has had the result that populations are no longer routinely vaccinated against these infectious agents, and the increasing emergence of antibiotic-resistant bacteria, humans are susceptible to acute outbreaks of disease or to biological terrorism. Antibiotics are obviously ineffective at eliminating viral infections, and the antiviral drugs in use today are often associated with a short serum half-life and resistance often emerges after repeated use[21]. Passive antibody serum therapy is today used merely in replacement therapy for patients with immune disorders, for POST-EXPOSURE PROPHYLAXIS against several viruses (for example, rabies, measles, hepatitis A and B, varicella and respiratory syncitial virus (RSV)) and for toxin neutralization (for example, diphtheria, botulism and tetanus). Passive immunization has substantial advantages over the administration of antimicrobial agents, including low toxicity and high specific

EFFECTOR FUNCTIONS
The antigen-elimination processes mediated by immunoglobulins and initiated by the binding of effector molecules to the Fc part of the immunoglobulin. The common effector functions are complement-dependent cytotoxicity (CDC), phagocytosis and antibody-dependent cellular cytotoxicity (ADCC).

COMPLEMENT-DEPENDENT CYTOTOXICITY
Once bound to antigen, both IgM and IgG can trigger a sequence of reactions by which serum proteins called complement factors are cleaved. One of the results is destruction of the target cell through complement-dependent cytotoxicity.

PROGRAMMED CELL DEATH
Programmed cell death infers that cells are determined to die at a specific stage of development or having received a specific signal. The process is known as apoptosis. The cells shrivel and are engulfed by nearby phagocytic cells without eliciting any inflammatory response.

Box 1 | **Immunoglobulin classes**

Antibodies belong to either one of five immunoglobulin (Ig) classes: IgA, IgD, IgE, IgG or IgM. Each class has a distinct structure and biological activity. Some of the classes are further divided into subclasses — for example, there are four IgG subclasses and two IgA subclasses. IgM is the first antibody to be produced in an immune response and forms a pentameric complex comprised of Ig monomers. IgA is the main class of antibody in external secretions, where it is found as a dimer that protects the body's mucosal surfaces from infection; it is also found as a monomer in serum. IgD is the main antibody on the surface of B cells. IgE is found bound to cells that secrete histamines after antigen binding. IgG is the main antibody in serum. The IgG class is the most stable and has a serum half-life of 20 days, whereas IgM and IgA persist for only 5–8 days. Both IgM and IgG can mediate complement fixation, whereas only IgG can promote antibody-dependent cellular cytotoxicity (ADCC). IgA, and to a certain degree IgM, can mediate trancytosis to mucosal surfaces, whereas only IgG can be transported across placenta for fetal protection.

activity, as well as an immediate effect compared with vaccines and even antibiotics. Given these issues, biotechnology companies and institutions working within the field of infectious disease protection are certain to direct efforts towards developing highly effective and functional antibody candidates against specific disease targets.

The isolation of protective toxin-neutralizing human monoclonal antibodies was described in 1993 in a study in which several human monoclonal antibodies against tetanus toxin were isolated and a protective effect against tetanus toxin was observed[22]. However, when combinations of these specific monoclonal antibodies were administered, an extra potent (that is, synergistic) effect was observed. Such an effect has also recently been described in the neutralization of botulinum neurotoxin. The anti-botulinum toxin antibodies were derived from different phage-display libraries obtained from humans or immunized mice (J. Marks, presented at Cambridge Healthtech Institute conference on Recombinant Antibodies, Cambridge, Massachusetts, USA, 24–25 April 2002). Recently, Maynard and colleagues described high-affinity antibodies against *Bacillus anthracis*[23]. These antibodies were fragments derived from variable-chain genes of a mouse monoclonal antibody and expressed in *E. coli* as single-chain Fv fragments (scFvs). By administering the antibody fragments to mice before injection of anthrax toxin, protection against the toxin was observed. Protective human antibodies against Shiga-toxin1 were recently isolated by the immunization of transgenic mice[20]. The panel of ten different antibodies of the IgM and IgG1 class that showed specificities to different subunits of the toxin effectively neutralized the toxin.

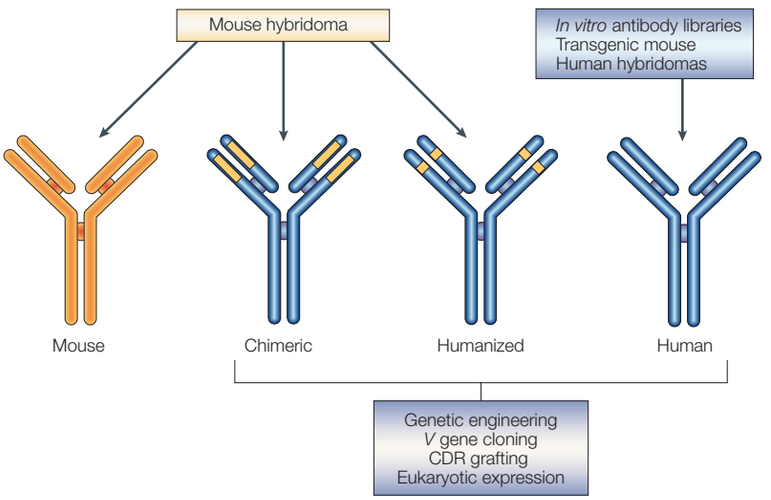

Figure 2 | **Antibody engineering.** Mouse hybridoma technology generates mouse monoclonal antibodies. Genetic engineering has fostered the generation of chimeric, humanized and human antibodies. Cloning of mouse variable genes into human constant-region genes generates chimeric antibodies. Humanized antibodies are generated by the insertion of mouse complementarity-determining regions (CDRs) onto human constant and variable domain frameworks; however, additional changes in the framework regions have, in several cases, been shown to be crucial in maintaining identical antigen specificity[75,76]. Fully human antibodies can be generated by the selection of human antibody fragments from *in vitro* libraries (see BOX 2 and FIG. 3a), by transgenic mice (FIG. 3b) and through selection from human hybridomas.

Compared with toxin neutralization, the use of antibodies in the prevention of viral disease is a more complex prospect. Palivizumab (Synagis; MedImmune Inc) is a humanized IgG1 monoclonal antibody approved for the prevention of RSV infections in high-risk infants[17,24]. Palivizumab was the first monoclonal antibody approved for an infectious pathogen and is, so far, the only antiviral monoclonal antibody in clinical use. The development of sevirumab (Protovir; Protein Design Labs) — a human anti-cytomegalovirus (CMV) antibody — was, by contrast, halted in Phase III clinical trials as a supplemental treatment for CMV-induced retinitis because of a lack of evidence of efficacy. The elimination of a viral infection requires that a number of events occur, including inhibition of cell infection, mediation of cell killing of infected cells, inhibition of viral replication, inhibition of viral release and inhibition of cell–cell transmission[25,26]. Within the multitude of antibody specificities generated in the typical human polyclonal response, it is likely that one or several effective antibodies against one or more of the particular processes listed will be found. Some of these antibodies bind neutralizing epitopes, whereas others bind non-neutralizing epitopes. The overall outcome might indeed be effective protection due to the combination of the blocking, neutralizing and eliminating effect of human antibodies. Recently, XTL Pharmaceuticals reported on Phase I/II clinical trials with two human monoclonal antibodies against hepatitis B virus[28]. The two monoclonal antibodies were combined with lamivdudine (Epivir-HBV; GlaxoWellcome) — an antiviral drug that inhibits DNA replication — and showed significant reduction in serum viral titre. If monoclonal antibodies are to be used as prophylactics or therapeutics against infectious diseases, it is likely that their efficacy will be increased when a polyclonal passive human serum therapy is mimicked — that is, when a pool of highly specific and high-affinity monoclonal antibodies are administered. There are, however, limitations to this approach, including the production costs associated with manufacturing intact human antibodies.

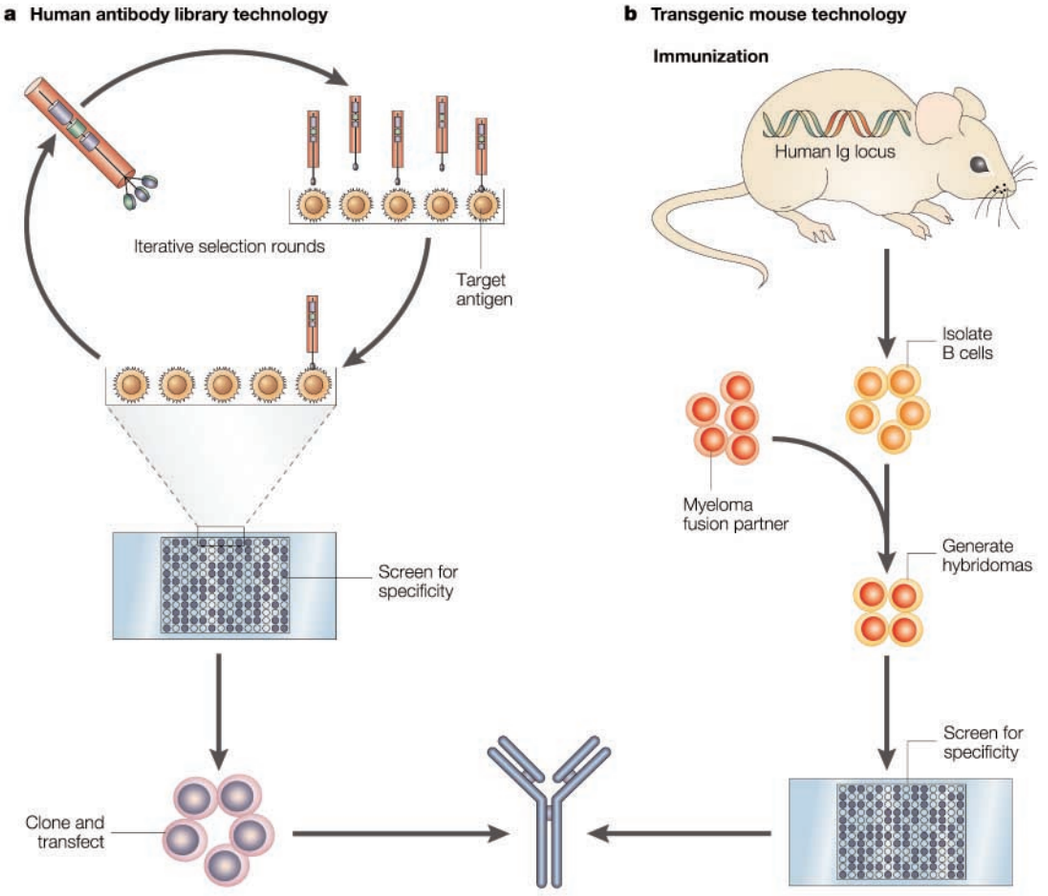

Figure 3 | ***In vitro* and *in vivo* human antibody techniques exemplified by phage display and transgenic mouse technologies. a** | The *in vitro* process is based on panning the library of antibodies against an immobilized target. The non-binding phage antibodies are washed away and the recovered antibodies are amplified by infection in *Escherichia coli*. The selection rounds are subsequently repeated until the desired specificity is obtained. The antibody format for screening is either Fab or single-chain Fv. The expression of antibodies in *E. coli* and recent developments in screening technologies[77] have made it possible to screen tens of thousands of clones for specificity. The antibody fragments themselves can be used as therapeutic agents as discussed in this review, but they can also be converted into intact immunoglobulins by the cloning of the variable genes into plasmids incorporating the constant-region genes of immunoglobulins. The genes are transfected into cell lines and therefore produce fully human immunoglobulins. **b** | The *in vivo* process is based on the immunization of a transgenic mouse. The mouse has been genetically engineered and bred for the expression of human immunoglobulins. The B cells harvested after immunization can be immortalized by fusion with a myeloma cell line, as in traditional hybridoma technology. The hybridomas can then be screened for specific antibodies.

HYBRIDOMA
An antibody-secreting B-cell line that is generated by fusing splenic-derived B cells with a plasmacytoma. A hybridoma produces the same antibody as the parent B cell and divides and grows in culture like the parent cancer cell. The antibody produced is monoclonal.

SERUM THERAPY
The treatment of an infectious disease with the serum from an immunized animal or individual, and which contains antibody.

POST-EXPOSURE PROPHYLAXIS
A treatment that is designed to protect an individual against a disease agent to which the individual has been recently exposed.

A possible solution to this problem is to instead use blocking antibody fragments synthesized in *E. coli*, which could be used to combat, for example, viral infections. A further difficulty in developing such multi-antibody therapeutics might arise from regulatory concerns regarding the administration of multiple monoclonal antibodies. The experience obtained from XTL Pharmaceuticals' use of combined antibodies might be an important step in resolving — or exacerbating — these concerns.

Anti-inflammatory antibodies

Antibodies with high specificity and affinity can be developed to bind specific cytokines or their receptors. In both cases, the purpose is to inhibit the detrimental effect of the cytokine. Cytokines associated with inflammation and autoimmunity include tumour-necrosis factor-α (TNF-α), interleukins and complement proteins. Modulation of immune-responses, such as immune-cell depletion by the targeting of antibodies to cell-surface receptors, for example, CD20 and CD4 on B or T cells, has also been shown as a viable therapeutic strategy in autoimmune diseases. Together with cancer, inflammatory and autoimmune diseases are an important focus for companies developing antibody therapies. In patients with rheumatoid arthritis (RA), TNF-α accumulates in the joints and contributes to the inflammation and joint destruction that is associated with the disease. Marketed products directed towards the regulation of TNF-α include the soluble TNF-α receptor eternacept (Enbrel; Amgen Inc/Wyeth) and the antibody infliximab

Table 1 | **Approved monoclonal antibodies**

Product	Year approved	Type of molecule	Disease indication	Company
OKT-3	1986	Murine (anti-CD3)	Organ transplant rejection	Ortho Biotech Products LP
ReoPro	1994	Chimeric (anti-platelet gpIIb/IIIa)	Coronary intervention and angioplasty	Centocor Inc
Panorex (Germany only)	1995	Murine (anti-EpCAM)	Colorectal cancer	Centocor Inc
Rituxan	1997	Chimeric (anti-CD20)	Non-Hodgkin's lymphoma	IDEC Pharmaceuticals Corp
Zenapax	1997	Humanized (anti-IL-2 receptor)	Refractory unstable angina	Centocor Inc
Herceptin	1998	Humanized (anti-ERBB2)	Metastatic breast cancer	Genentech Inc
Remicade	1998	Chimeric (anti-TNF-α)	Crohn's disease	Centocor Inc
Simulect	1998	Chimeric (anti-IL-2 receptor)	Kidney transplant rejection	Novartis AG
Synagis	1998	Humanized (anti-F-protein)	Respiratory syncitial viral disease	MedImmune Inc
Mylotarg	2000	Humanized (anti-CD33)	Chemotherapy for acute myeloid leukemia	Celltech Group plc/Wyeth
Campath	2001	Humanized (anti-CD52)	B-cell chronic lymphocytic leukemia	Millennium Pharmaceuticals/ Ilex Oncology Inc
Zevalin	2002	Mouse (anti-CD20)	B-cell non-Hodgkin's lymphoma	IDEC Pharmaceuticals Corp
Xolair (Australia only)	2002	Humanized (anti-IgE Fc)	Allergy	Tanox Inc/Genentech Inc/ Novartis AG

Adapted and updated from REF. 81.

(Remicade; Centocor Inc). Infliximab is a chimeric antibody with mouse variable domains and human constant domains of the IgG1 subclass. There are a number of anti-TNF-α antibodies in clinical trials, including CDP571 and CDP870 (Celltech Plc) and adalimumab (D2E7; Abbot Laboratories/Cambridge Antibody Technology). Adalimumab is the first phage-display-derived human antibody brought into the clinic, and was generated by 'guided selection' using a mouse monoclonal antibody[28]. The method is based on the selection of a human variable-domain repertoire coupled to one of the original mouse variable domains, so as to 'guide' the human variable-domain repertoire towards the same specificity as the original mouse variable domains. The antibody is affinity optimized by iterative rounds of selection and mutagenesis (FIG. 3; BOX 2, 3). Adalimumab has completed Phase III clinical trials and is currently in registration for FDA approval. Recently, the anti-CD20 antibody rituximab (Rituxan; IDEC Pharmaceuticals; see below) completed Phase II clinical trials in RA and showed promising results compared with the anti-TNF-α antibodies adalimumab and infliximab[29], indicating that depletion of B cells might be an effective treatment in RA and other autoimmune diseases[30]. Eculizumab (5G1.1; Alexion Pharmaceuticals) is a humanized monoclonal antibody that prevents the cleavage of human complement component C5 into its pro-inflammatory components[31], whereas J695 (Cambridge Antibody Technology) is a human antibody derived from a phage-display library against interleukin-2 (see online links box). Both antibodies are in clinical trials at present and have shown potential in the treatment of inflammatory diseases such as RA and nephritis.

Antibodies targeting cancers

In cancer therapy, the purpose of antibody administration is to induce the direct or indirect destruction of cancer cells, either by specifically targeting the tumour or the vasculature that nourishes the tumour. Indeed, new technologies for the panning of antibody libraries on intact cells have made it possible to isolate antibodies against novel and promising cancer-associated antigens[32]. However, the most common cancer targets are the carcinoembryonic antigen (CEA), which is associated with colorectal cancers, MUC1, epidermal growth factor receptor (EGFR) and ERBB2 (also known as HER2/neu, associated with lung and breast cancer) and CD20 on B cells, which is a marker for non-Hodgkin's lymphoma (NHL). Examples of approved therapeutic antibodies directed towards ERBB2 and CD20 include the humanized IgG1 antibody trastuzumab (Herceptin; Genentech) for breast cancer and the chimeric IgG1 antibody rituximab (Rituxan; IDEC Pharmaceuticals) for NHL.

Today, specific antibody therapy is used in combination with classical chemotherapy, but the remaining challenge is to develop specific and highly cytotoxic drugs against cancer cells. Recently, the FDA approved two new antibody chemotherapies: ibritumomab tiuxetan (Zevalin; IDEC Pharmaceuticals) and gemtuzumab ozogamicin (Mylotarg; Wyeth). Ibritumomab tiuxetan is a mouse anti-CD20 antibody

attached to the radioisotope ^{90}yttrium that targets the surface of the mature B cells and B-cell tumours, inducing cellular damage in the target and neighbouring cells. Ibritumomab tiuxetan is the first radioimmunotherapeutic antibody approved by the FDA and is approved for use in the treatment of NHL. The antibody reacts with the same antigen as rituximab, but is a mouse antibody, which results in beneficial rapid clearance to decrease the undesired effect of total body irradiation. Gemtuzumab ozogamicin is a humanized monoclonal antibody that is linked to the antitumour agent calicheamicin, a bacterial toxin[33]. The antibody is targeted to CD33, which is expressed in about 90% of all acute myelogenic leukaemia (AML) cases, and has been approved for administration to patients who have relapsed AML. Both ibritumomab tiuxetan and gemtuzumab ozogamicin are examples of antibodies designed to specifically deliver their toxic load directly to cancer cells. Antibodies directed against major histocompatibility complex (MHC) class II proteins specifically target and eliminate cancer cells. The mouse monoclonal antibody Oncolym (Lym-1; Peregrine Pharmaceuticals)[34] and the antibody Hu1D10 (Remitogen; Protein Design Labs)[35] are two examples of such antibodies. Whereas one form of Oncolym is radiolabelled with ^{131}iodine (REF. 36), the humanized IgG1 Hu1D10 eliminates cancer cells by virtue of its natural effector functions (FIG. 4 and see below), and also induces apoptosis. Recently, an anti-MHC class II human antibody derived from an antibody phage-display library was shown to induce apoptosis of activated lymphoid cells[19]. Vascular endothelial growth factor (VEGF) is a potent cytokine for angiogenesis (the formation of blood vessels). Inhibitory agents, including specific antibodies, have been developed to block VEGF-stimulated angiogenesis as a strategy to inhibit tumour growth. One example of such an antibody is the humanized anti-VEGF antibody bevacizumab (Avastin; Genentech), which is in Phase III clinical trials for the treatment of breast cancer and colorectal cancer. Monoclonal antibodies are being continuously developed for cancer therapy, and most new antibody formats have demonstrated utility within this field.

Naked antibodies

Both trastuzumab and rituximab are 'naked antibodies', meaning that they do not have a radioisotope or toxin attached to them. The elimination of the target of these antibodies depends entirely on the recruitment of the body's own effector mechanisms, namely complement

Table 2 | **A selection of antibodies in clinical development**

Product	Stage (2002)	Type of molecule	Disease indication	Company
ABX-EGF	Phase II	Human (anti-EGF-R)	Non-small-cell lung cancer	Abgenix Inc/Immunex Corp
ABX-IL8	Phase II	Human (anti-IL-8)	Pulmonary disease, chronic obstructive bronchitis	Abgenix Inc/Immunex Corp
Eculizumab (5G1.1)	Phase IIb	Humanized (anti-C5)	Rheumatoid arthritis, lupus nephritis	Alexion Pharmaceutical Inc
D2E7	Phase III	Human (anti-TNF-α)	Rheumatoid arthritis	Cambridge Antibody Technology/ Abbot Laboratories
CAT-152	Phase II/III	Human (anti-TGF-β2)	Scarring following glaucoma surgery	Cambridge Antibody Technology
J695	Phase II	Human (anti-IL-2)	Autoimmune diseases including rheumatoid arthiritis	Cambridge Antibody Technology/Abbot/ Wyeth Genetics Institute
Antegren (natalizumab)	Phase III	Humanized (anti-α-4 integrin)	Crohn's disease, multiple sclerosis, inflammatory bowel disease	Elan Pharmaceuticals Corp/Biogen Inc
Avastin (rhuMAb-VEGF)	Phase III (anti-VEGF)	Humanized	Metastatic breast cancer, non-small-cell lung cancer	Genentech Inc
IDEC-151 (Clenoliximab)	Phase II	Primatized (anti-CD4)	Rheumatoid arthritis	IDEC Pharmaceuticals Corp
MEDI-507 (Siplizumab)	Phase II	Humanized (anti-CD2 Receptor)	Suppresses NK and T-cell function	MedImmune Inc
XTL 001 (pairs of monoclonals)	Phase I/II	Human (anti-HBV)	Hepatitis B virus neutralization	XTL Bioharmaceuticals Ltd
CDP870	Phase II	Fab fragment (anti-TNF-α)	Rheumatoid arthritis	Celltech Group plc
CDP571	Phase III	Humanized (anti-TNF-α)	Crohn's disease	Celltech Group plc
Hu1D10 (Remitogen)	Phase II	Humanized (anti-MHC class II)	Non-Hodgkin's Lymphoma	Protein Design Labs Inc

Adapted from REFS 34, 81 and selected company web sites.

Box 2 | *In vitro* human antibody libraries

The strategy of selecting antibodies from large repertoires is dependent on the coupling between genotype and phenotype — that is, the displayed protein (the antibody) carries its own encoding gene. As a result, the process enables the easy recovery of the DNA encoding the selected protein. The power of such antibody libraries lies in the fact that selection takes place *in vitro*, thereby making them excellent systems for isolating antibodies to certain drugs, potent toxins and haptens that would normally be impossible to raise within *in vivo* systems such as the mouse hybridoma technology.

In phage display, the variable genes encoding the antibody variable domains are fused to genes encoding bacteriophage coat proteins. The most commonly used phage protein is the pIII protein located at the tip of the long, thin filamentous phage M13. The system is highly effective and is used to isolate single-chain Fv or Fab fragments with specificity to almost any kind of antigen. The plasmid encoding the variable genes is packaged within the viral capsid and the expressed antibody is presented on the bacteriophage surface. Phage libraries are made from the human naive repertoire (that is, the IgM and IgD class) from pre-immunized donors or totally synthetic variable genes. As the repertoire is created *in vitro*, the technology also generates high-affinity human antibodies against human proteins. The process is based on panning the library against an immobilized target in a test tube (FIG. 3a). The non-binding phage antibodies are washed away and the recovered antibodies are amplified by infection in *E. coli*. The selection rounds are subsequently repeated until the desired specificity is obtained.

In messenger RNA (mRNA)-display technologies, such as the ribosomal display technology, antibody domains are linked to ribosomes attached to the mRNA that they are translating. This process takes place entirely *in vitro* by transcripton of a DNA library followed by *in vitro* translation of the mRNA library. The subsequently recovered mRNA transcripts are reverse transcribed and amplified by polymerase chain reaction (PCR) for the subsequent round of selection. The method has been successfully used for the affinity maturation and stability engineering of antibody fragments[58,70]. A similar technology called ProFusion is based on the covalent linkage of the synthesized proteins to the mRNA template via a puromycin linker[71]. Another potential display method applicable for antibody display is the Covalent Display Technology. In this method, the protein is fused to P2A, a bacteriophage DNA-nicking protein that covalently binds its own DNA[72].

activation and Fc-receptor-dependent responses (FIG. 4). Such effector functions can be abolished or augmented by mutagenesis of the Fc part of the antibody. Both trastuzumab and rituximab seem to exert their effects primarily through an Fc-receptor-dependent mechanism[37], and both are of the IgG1 subclass of antibodies, and thus bind all known Fcγ receptors (FcγRs). These receptors are located in the membrane of various effector cells, such as natural-killer (NK) cells, neutrophils, monocyte/macrophages, dendritic cells and B cells. In general, IgG-FcγR crosslinking leads to antibody-cell cytotoxicity or phagocytosis of the target cells by the effector cell (FIG. 4). In addition, depending on the nature of the target, the crosslinking caused by FcR interaction can itself induce signalling that leads to apoptosis. Although most of the FcγRs exert the functions mentioned above, one class of FcγRs, FcγRIIb, has a general inhibitory effect. Recently, a thorough investigation of the interaction between IgG and FcγRs led to the discovery of IgG1 variants with improved or decreased binding affinity for the FcγRs, including some with increased binding affinity for the activating receptors and decreased binding to the inhibiting ones[38]. So, variants can now be selected with desired combinations of functions.

Another approach to enhance the effector functions of antibodies is to engineer bispecific antibodies, which comprise two specificities, one for the cell to be eliminated and one for receptors on effector cells such as cytotoxic T cells (BOX 4). Strategies for the production of bispecific antibodies have been developed[39], and an important new approach in this field is the activation of polymorphonuclear leucocytes (PMNLs), the most numerous cytotoxic effector-cell population. These cells have Fc receptors for the antibody class IgA (Fcγ receptor, CD89) and this has elicited a growing interest in IgA[40], and bispecific antibodies that recruit PMNLs through CD89 binding have shown promise in *in vitro* studies[41].

A possible side effect of antibody therapy is the cytokine-release syndrome that is probably mediated through recruitment of the immune effector cells.

Box 3 | Affinity maturation of antibodies

In the mammalian immune system, antibodies undergo affinity maturation and through the development of memory B cells retain an antibody repertoire for subsequent encounter of an identical pathogen. Similarly, *in vitro* antibody technologies have enabled the selective alteration of the affinity of antibodies. Several approaches have been developed, but a common element to all of these is that they are developed under stringent selection regimes, including reducing the concentration of antigen, altering washing conditions (see BOX 2), changing temperature or eluting with free antigen. Methods of antibody affinity maturation are based on the principle of changing parts of, or the whole, antibody variable domains while simultaneously keeping the specificity. Most of the contribution of specific antibody binding resides within the variable heavy-chain domain and especially within the CDR 3 loops of both variable heavy and variable light chains (see FIG. 1).

Chain-shuffling is based on the method of substituting the native light chain with a new light-chain repertoire, but retaining the variable heavy chain. Panning of such chain-shuffled libraries in stringent conditions generates antibodies with higher affinities than the mother antibody clone. The randomization of CDR regions has also been used as a maturation strategy. Another approach is to generate an antibody library with mutations within the variable genes either by error-prone polymerase chain reaction, *E. coli* mutator strains or site-specific mutagenesis. Both phage display and ribosomal display have been successfully used for affinity maturation.

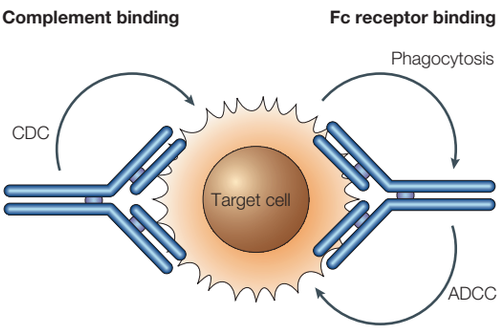

Figure 4 | **Natural immunoglobulin effector functions.** The classical complement pathway is initiated by the binding of C1 to immune complexes (that is, antibody-coated cells or antigens) and eventually leads to cellular lysis and elicits inflammatory responses for the ultimate elimination of the target cell. This process is called complement-dependent cytotoxicity (CDC). The Fc-receptor-mediated effector functions are associated with the recruitment of cellular effectors leading to either phagocytosis or antibody-dependent cellular cytotoxicity (ADCC). These mechanisms are accepted to be the processes that operate when naked antibodies are used as therapeutic agents.

In such cases, abrogation of Fc-dependent effector functions is desired and this can be achieved by mutating single amino acids in the Fc region of the antibodies[42].

Antibodies without Fc

Small antibody-derived fragments can do part of the job normally performed by intact antibodies, such as blocking the action of a toxin — for example, anthrax toxin[23] — or blocking the interaction between a cytokine and its receptor[43,44]. These antibody fragments can also carry effector molecules to their targets. The blocking effect of antibodies simply requires the inhibition of subsequent ligand or receptor binding, and does not necessarily need effector functions. However, even fragments with effector functions normally associated with 'naked antibodies' — the bispecific antibody fragments — have been described. These fragments comprise either the Fv or Fab portion of the antibody (FIG. 1), which in both cases is the part that binds antigen. Such antibody fragments can be functionally expressed in *E. coli* with ease, which is a prerequisite for antibody phage display as well as for other *in vitro* human antibody library approaches. One of the great advantages of the library approaches in general is that they allow for the selection of antibodies of high specificity and affinity towards a variety of different antigens (BOX 2). Engineered antibodies have therefore become smaller, with increasing affinity and specificity. Another obvious advantage is the ability to select specific antibodies against toxins, drugs, cytokines and other targets that cannot, for various reasons, be injected into an animal to raise an immune response, one of which is that the target might kill the animal or that it might not be immunogenic at all (BOX 2).

Coupling the antibody fragments into dimers and trimers generates oligovalent antibody fragments with increased avidity and the ability to crosslink target molecules (FIG. 5). In this approach, the antibody is first reduced to the small antigen-binding portion and then engineered to form a larger molecule to gain avidity and crosslinking ability. So, the signalling effect that requires crosslinking of receptors might also be achieved with antibody fragments. The so-called triabodies are antibody fragments in which the valency is increased to three antigen-binding sites[45,46], thereby increasing the avidity of the antibody fragments. Such multivalent antibody fragments might prove to be effective in the blocking and neutralization of toxins, as well as in the generation of targeting devices for cancer therapy.

Antibody fragments with effector functions normally associated with 'naked antibodies' — the diabodies — were first described in 1993 (REF. 47). Diabodies are scFv fragments with two different antigen specificities, one directed against the target and one directed against effector molecules such as complement[48] or Fc receptors[49]. Since then, diabodies have been designed in various ways to crosslink effector cells such as cytotoxic T cells[50,51] and NK cells[49], which is particularly useful for the treatment of lymphomas. Tandem diabodies[52], in which two diabodies are genetically coupled to achieve a better crosslinking effect, are still at an early stage of development. Another early-stage antibody format is pepbodies[53], which comprise Fab or scFv fragments linked to small peptides that bind effector molecules and thus mimic the way complete antibodies bind their effector molecules.

Antibody pharmacokinetics

A prerequisite for effective antibody targeting is that the antibodies should be able to penetrate tissues, and small antibody fragments are better than complete antibodies in this respect. Recent research has shown that high-affinity fragments are retained in the periphery of the tumour, whereas the medium-affinity antibodies penetrate throughout the tumour[54]. Furthermore, bivalent low-affinity fragments penetrate better and more uniformly than high-affinity fragments[55]. Fortunately, antibody fragments can be selected for optimal affinity and specificity by all of the *in vitro* selection processes[56,57]. The therapeutic efficacy of an antibody, however, also depends on its stability as well as its immunogenicity. Strategies for the engineering of antibody stability with regards to antibody fragments have been described[58].

Box 4 | **Strategies for making bispecific antibodies**

Bispecific antibodies have the ability to bind two different antigens and have been used to crosslink various cells and molecules, although they have been primarily used to re-direct effector cells to target cells (FIG. 5). Methods for generating bispecific antibodies tend to bias the production towards heterodimers — that is, towards two specificities instead of one. The first method was described in 1983 (REF. 73) in the generation of so-called hybrid-hybridomas, or quadromas, and is based on the fusion of two hybridoma cell lines, but this is a complex and time-consuming effort; chemical or genetic coupling of Fab fragments[74] is more effective. The inclusion of a C_H3 domain into antibody fragments has been used to function as a dimerization domain, and a modified form of a C_H3 domain has been used to direct hetero-dimerization[39]. Perhaps the most effective way to generate bispecific antibody fragments is by expression of bispecific single-chain Fv fragment as diabodies (FIG. 5).

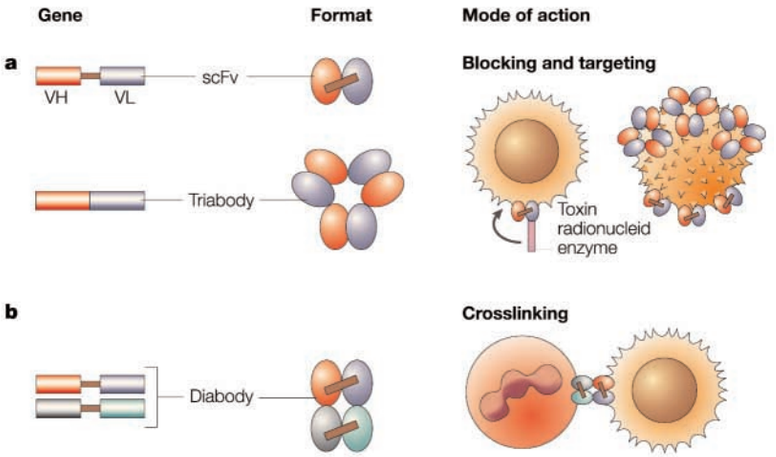

Figure 5 | **Different formats of antibody fragment. a** | Single-chain Fv fragments (scFvs) are mostly monomeric when the peptide linker (in red) between the V_H and the V_L domains is about 12–15 amino acids. When the linker is shorter than three amino acids or is totally deleted, the fragments tend to make trimeric fragments or even tetrameric formats. Multivalent antibodies gain avidity and such molecules are therefore perfect for blocking viral infection or receptor–ligand interactions, for example. **b** | If the linker is about five amino acids long, then the variable domains tend to dimerize. When equipped with variable domains with different specificities, such dimeric scFvs can make bispecific fragments called diabodies.

Similarly, the presumed immunogenicity of human antibodies can be reduced by mutagenesis of typical B-cell[59] or T-cell epitopes[60]. As the development of monoclonal antibodies moved away from mouse antibodies to chimeric antibodies and subsequently to humanized antibodies, a reduction in the level of anti-antibody responses was observed with new antibody products. It will, however, be interesting to observe the immunogenicity of fully human antibodies in therapeutic settings. In particular, a comparison of D2E7 with infliximab (that is, a human versus a chimeric anti-TNF-α monoclonal antibody) will provide valuable information in this respect. Small antibody fragments show rapid clearance from the circulation, and as a result the fraction of the injected dose that reaches its target is at present too low for a therapeutic benefit, even for bivalent fragments. Therefore, the manipulation of antibody fragments to increase their half-life will be crucial for the success of therapeutic antibody fragments.

Complete human antibodies have long serum half-lives owing to their ability to bind to the so-called neonatal Fc receptor (FcRn). This receptor both transports IgG across the placenta from mother to fetus and protects serum IgG from degradation. FcRn is expressed on placenta and blood-vessel linings and binds IgG in the junction between the C_H2 and C_H3 domains of the Fc region[61] (see FIG. 1). As Fab and scFv fragments lack the Fc region altogether, they are not rescued from degradation by this mechanism and, as a result, have a short serum half-life.

A new approach to increasing the half-life of antibodies is the PEGYLATION of antibodies and antibody fragments, which simultaneously reduces their immunogenicity[62]. The effect is achieved by chemical coupling of polyethylene glycol (PEG) to amino groups in the protein structure. The main property contributing to this effect is the increase in the size of the molecule above the glomerular-filtration limit. However, modifications of amino groups within antibodies affect the effector functions[63] as well as the antigen-binding capacity[64]. A possible alternative is to direct the PEG attachment to sites on the antibody molecules that are distant from the CDR regions and crucial sites for effector functions[62.] Indeed, an anti-TNF-α human Fab fragment (CDP 870; Celltech) has its circulating half-life prolonged to 14 days by site-specific pegylation in the hinge region[44].

PEGYLATION
The covalent attachment of the polymeric molecule polyethylene glycol (PEG) to proteins.

TROYBODY
An antibody with specificity for an antigen-presenting cell and with an antigenic fragment inserted into a constant domain.

The potentiation of immune responses

Antibodies are the perfect high-specificity targeting and delivery devices. However, in the immune responses mounted by the body in response to pathogenic organisms and cancer, the cellular response mediated by T cells is a crucial component. Infectious diseases are eliminated by the combined action of antibodies and T cells. In the search for optimized vaccines, the aim is to use agents that both stimulate antibody and T-cell responses. A discussion of the immune effects of T cells is beyond the scope of this review, although new antibody-based technologies that aim at modulating T-cell responses are important issues for the potentiation of a desired therapeutic effect by, for example, therapeutic antibodies. One strategy for increasing the immune response is to load antibodies with antigenic material in the CDR regions[65]. This strategy is one of several that aim to use the Fc portion of the antibody to load specialized immune cells with antigenic material. In the TROYBODY strategy[66], the antibodies carry pieces of antigenic material as part of their Fc region and target the specialized immune cells by way of their Fab regions. In all cases, the purpose of the immune-cell loading is to activate disease-specific helper T cells. Indeed, Troybodies increase such T-cell responses more than 10,000-fold compared with the administration of the antigenic material in free form[67,68]. This strategy requires that the antigenic material is characterized, which is a significant challenge. An advantage of this approach, however, is that it can be combined with strategies that aim at activating the cytotoxic T cells that are crucial in the destruction of cancer and virus-infected cells. Recently, myeloma cells were coated with antibodies specific for syndecan-1 (SDC1), an antigen that is highly expressed on these tumour cells, which promoted the uptake of the tumour cells by dendritic cells through an Fc-receptor-dependent mechanism and the subsequent stimulation of tumour-specific cytotoxic T cells[69].

Concluding remarks

Throughout the development of therapeutic antibodies over the past 30 years, many obstacles have been overcome and, today, therapeutic antibodies have entered a new era. It is no longer a significant effort to select human antibodies against any kind of disease target. The first antibody derived from phage antibody

libraries has recently completed Phase III trials, and there are, at the moment, several other antibodies derived from both *in vitro* libraries and transgenic mice in clinical trials. There is a rising awareness of antibody effector functions and efforts are being directed towards modulating Fc-associated functions, such as complement activation and Fc-receptor activity. A remaining impediment to the development of new antibody therapeutics by the pharmaceutical industry is the cost of producing antibodies with mammalian expression systems. The new small therapeutic antibody formats, such as scFv, Fab or diabodies, can, however, be produced in *E. coli* at a much lower cost, and even antibody production in plants is a growing field. As antibodies are such versatile and highly specific molecules, fine tuned by millions of years of evolution to be optimal agents for targeted disease elimination, their development for various indications will, no doubt, be pursued with vigour for the foreseeable future.

1. Köhler, G. & Milstein, C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* **256**, 495–497 (1975).
2. Larrick, J. W. & Fry, K. E. Recombinant antibodies. *Hum. Antibodies Hybridomas* **2**, 172–189 (1991).
3. Khazaeli, M. B., Conry, R. M. & LoBuglio, A. F. Human response to monoclonal antibodies. *J. Immunother.* **15**, 42–52 (1994).
4. Morrison S. L., Johnson M. J., Herzenberg, L. A. & Oi, V. T. Chimeric human antibody molecules: mouse antigen-binding domains with human constant region domains. *Proc. Natl Acad. Sci. USA* **21**, 6851–6855 (1984).
5. Boulianne, G. L., Hozumi, N. & Shulman, M. J. Production of functional chimaeric mouse/human antibody. *Nature* **312**, 643–646 (1984).
6. Bell, S. & Kamm, M. The clinical role of anti-TNFα antibody treatment in Crohn's disease. *Aliment. Pharmacol. Ther.* **14**, 501–514 (2000).
7. Jones, P. T., Dear, P. H., Foote, J., Neuberger, M. S. & Winter, G. Replacing the complementarity-determining regions in a human antibody with those from a mouse. *Nature* **321**, 522–525 (1986).
8. Skerra, A. & Plückthun, A. Assembly of a functional immunoglobulin F_v fragment in *Escherichia coli*. *Science* **240**, 1038–1041 (1988).
9. Better, M., Chang, C. P., Robinson, R. R. & Horwitz, A. H. *Escherichia coli* secretion of an active chimeric antibody fragment. *Science* **240**, 1041–1043 (1988).
10. McCafferty, J., Griffiths, A. D., Winter, G. & Chiswell, D. J. Phage antibodies: filamentous phage displaying antibody variable domains. *Nature* **348**, 552–554 (1990).
11. Green, L. L. Antibody engineering via genetic engineering of the mouse: XenoMouse strains are a vehicle for the facile generation of therapeutic human monoclonal antibodies. *J. Immunol. Methods* **231**, 11–23 (1999).
12. Cragg, M. S., French, R. R. & Glennie, M. J. Signalling antibodies in cancer therapy. *Curr. Opin. Immunol.* **11**, 541–547 (1999).
13. Farah, R. A., Clinchy, B., Herrera, L. & Vitetta, E. S. The development of monoclonal antibodies for the therapy of cancer. *Crit. Rev. Eukaryotic Gene Expr.* **8**, 321–356 (1998).
14. Berard, J. L. A review of interleukin-2 receptor antagonists in solid organ transplantations. *Pharmacotherapy* **19**, 1127–1137 (1999).
15. Maini, R. *et al*. Infliximab (chimeric anti-tumour necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concominant methotrexate: a randomised phase III trial. *Lancet* **254**, 1932–1939 (1999).
16. Sandborn, W. J. & Hanauer, S. B. Antitumor necrosis factor therapy for inflammatory bowel disease: a review of agents, pharmacology, clinical results and safety. *Inflamm. Bowel Dis.* **5**, 119–133 (1999).
17. Saez-Llorens, X. *et al*. Safety and pharmacokinetics of an intramuscular humanised antibody monoclonal antibody to respiratory syncitial virus in premature infants with bronchopulmonary dysplasia. *Pediatr. Infect. Dis.* **17**, 787–791 (1998).
18. Huls, G. A. *et al.* A recombinant, fully human monoclonal antibody with antitumor activity constructed from phage-displayed antibody fragments. *Nature Biotechnol.* **17**, 276–280 (1999).
19. Nagy, Z. A. *et al.* Fully human, HLA-DR-specific monoclonal antibodies efficiently induce programmed death of malignant lymphoid cells. *Nature Med.* **8**, 801–807 (2002).
20. Mukherjee, J. *et al*. Production and characterization of protective human antibodies against shiga toxin 1. *Infect. Immun.* **70**, 5896–5899 (2002).
21. Zeitlin, L., Cone, R. A., Moench, T. R. & Whaley K. J. Preventing infectious disease with passive immunization. *Microbes Infect.* **2**, 701–708 (2000).
22. Lang, A. B., Cryz, S. J. Jr, Schurch, U., Ganss, M. T. & Bruderer, U. Immunotherapy with human monoclonal antibodies. Fragment A specificity of polyclonal and monoclonal antibodies is crucial for full protection against tetanus toxin. *J. Immunol.* **151**, 466–472 (1993).
23. Maynard, J. A. *et al.* Protection against anthrax toxin by recombinant antibody fragments correlates with antigen affinity. *Nature Biotechnol.* **20**, 597–601 (2002).
24. Groothuis, J. R., Nishida, H. Prevention of respiratory syncytial virus infections in high-risk infants by monoclonal antibody (palivizumab). *Pediatr. Int.* **44**, 235–241 (2002).
25. Parren, P. W. H. I. & Burton, D. R. The anti-viral activity of antibodies *in vitro* and *in vivo*. *Adv. Immunol.* **77**, 195–262 (2001).
26. Burton, D. R. Antibodies, viruses and vaccines. *Nature Rev. Immunol*. **2**, 706–713 (2002).
An excellent review on the use of antibodies against viral diseases and the use of donor antibodies as tools for the development of recombinant vaccines, a process termed 'reverse vaccinology'.
27. Eren, R. *et al.* Clinical evaluation (phase I) of a combination of two human monoclonal antibodies to HBV: safety and antiviral properties. *Hum. Antibodies* **11**, 27–28 (2002).
28. Jespers, L. S., Roberts, A., Mahler, S.M., Winter, G. & Hoogenboom, H. R. Guiding the selection of human antibodies from phage display repertoires to a single epitope of an antigen. *Biotechnology* **12**, 899–903 (1994).
29. Haan, K. Product development: Rituxan's challenge in RA. *Biocentury* **10**, A1–A5. (2002).
30. Looney, R. J. Treating human autoimmune disease by depleting B-cells. *Ann. Rheum. Dis.* **61**, 863–866 (2002).
31. Kaplan, M. Eculizumab (Alexion). *Curr. Opin. Investig. Drugs* **3**,1017–1023 (2002).
32. Roovers, R. C., Van der Linden, E., De Bruine A. P., Arends, J. W. & Hoogenboom, H. R. Identification of colon tumour-associated antigens by phage antibody selction on primary colorectal carcinoma. *Eur. J. Cancer* **37**, 542–549 (2001).
33. Larson, R. A. *et al*. Antibody-targeted chemotherapy of older patients with acute myeloid leukemia in first relapse using Mylotarg (gemtuzumab ozogamicin). *Leukemia* **16**, 1627–1636 (2002).
34. Epstein, A. L. *et al*. Two new monoclonal antibodies, Lym-1 and Lym-2, reactive human with B-lymphocyte and derived tumors with immunodiagnostic and immunotherapeutic potential. *Cancer Res.* **47**, 127–135 (1987).
35. Gingrich, R. D., Dahle, C. E., Hoskins, K. F. & Senneff, M. J. Identification and characterization of a new surface membrane found predominantly on malignant B-lymphocytes. *Blood* **75**, 2375–2387 (1990).
36. deNardo, S. J. *et al*. Treatment of B-cell malignancies with ^{131}I Lym-1 monoclonal antibodies. *Int. J. Cancer Suppl.* **3**, 96–101 (1988).
37. Clynes, R. A., Towers, T. L., Presta, L. G. and Ravetech, J. V. Inhibitory Fc receptors modulate *in vivo* cytoxicity against tumor targets. *Nature Med.* **6**, 443–446 (2000).
38. Shields, R. L. *et al*. High resolution mapping of the binding site on human IgG1 for FcγRI, FcγRII, FcγRIII, and FcRn and design of IgG1 variants with improved binding to the FcγR. *J. Biol. Chem.* **276**, 6591–6604 (2000).
39. Carter, P. Bispecific human IgG by design. *J. Immunol. Methods* **248**, 7–15 (2001).
40. Dechant, M. & Valerius, T. IgA antibodies for therapy *Crit. Rev. Oncol. Hematol.* **39**, 69–77 (2001).
41. Peipp, M. & Valerius, T. Bispecific antibodies targeting cancer cells *Biochem. Soc. Transactions* **30**, 507–511 (2002).
42. Presta, L. G. Engineering antibodies for therapy. *Curr. Pharm. Biotechnol.* **3**, 237–256 (2002).
Whereas the focus for therapeutic antibodies has mostly been directed towards the antigen-binding part of antibodies, this paper describes the very important aspect of antibody effector functions and the modulation of the *in vivo* actions of antibodies.
43. Weir, A. N. C. *et al*. Formatting antibody fragments to mediate specific therapeutic functions. *Biochem. Soc. Transactions* **30**, 512–516 (2002).
44. Choy, E. H. *et al.* Efficacy of a novel PEGylated humanized anti-TNF fragment (CDP870) in patients with rheumatoid arthritis: a phase II double-blinded, randomized, dose-escalating trial. *Rheumatology* **41**, 1133–1137 (2002).
45. Iliades, P., Kortt, A. A. & Hudson, P. J. Triabodies: single chain Fv fragments without a linker form trivalent trimers. *FEBS Lett*. **409**, 437–441 (1997).
46. Kortt, A. A., Dolezal, O., Power, B. E. & Hudson, P. J. Dimeric and trimeric antibodies: high avidity scFvs for cancer targeting. *Biomol. Eng* **18**, 95–108 (2001).
47. Holliger, P., Prospero, T. & Winter, G. "Diabodies": small bivalent and bispecific antibody fragments. *Proc. Natl Acad. Sci. USA* **90**, 6444–6448 (1993).
The pivotal paper describing diabodies. Since the publication of this paper, the concept has been used and developed by many groups and has become a means for generating bispecific antibodies.
48. Kontermann, R. E., Wing M. G. & Winter, G. Complement recruitment using bispecific diabodies. *Nature Biotechnol.* **15**, 629–631 (1997).
49. Kipriyanov, S. M. *et al*. Synergistic antitumor effect of bispecific CD19 x CD3 and CD19 x CD16 diabodies in a preclinical model of non-Hodgkin's lymphoma. *J. Immunol*. **169**,137–144 (2002).
50. Holliger, P., Brissinck, J., Williams, R. L., Thielemans, K. & Winter, G. Specific killing of lymphoma cells by cytotoxic T-cells mediated by a bispecific diabody. *Protein Eng.* **9**, 299–305 (1996).
51. Cochlovius, B. *et al*. Treatment of human B cell lymphoma xenografts with a CD3 x CD19 diabody and T cells. *J. Immunol.* **165**, 888–895 (2000).
52. Kipriyanov, S. M. *et al.* Bispecific Tandem diabody for tumor therapy with improved antigen binding and pharmacokinetics. *J. Mol. Biol.* **293**, 41–56 (1999).
53. Lunde, E. *et al.* Troybodies and Pepbodies. *Biochem. Soc. Trans.* **30**, 500–506 (2002).
54. Adams, G. P. *et al*. High affinity restricts the localization and tumor penetration of single-chain fv antibody molecules. *Cancer Res.* **61**, 4750–4755 (2001).
55. Nielsen, U. B., Adams, G. P., Weiner, L. M. & Marks, J. D. Targeting of bivalent anti-ErbB2 diabody antibody fragments to tumor cells is independent of the intrinsic antibody affinity. *Cancer Res.* **60**, 6434–6440 (2000).
56. Schier, R. *et al.* Isolation of high-affinity monomeric human anti-c-erbB-2 single chain Fv using affinity-driven selection. *J. Mol. Biol.* **255**, 28–43 (1996).
57. Hanes, J., Schaffitzel, C., Knappik, A. & Plückthun, A. Picomolar affinity antibodies from a fully synthetic naive library selected and evolved by ribosome display. *Nature Biotechnol.* **18**, 1287–1292 (2000).
Describes the selection of antibody fragments with the highest affinities reported so far. This also validates the ribosomal-display technology (see Box 2) as a potential tool for affinity maturation of antibodies.
58. Wörn, A. & Plückthun, A. Stability engineering of antibody single-chain Fv fragments. *J. Mol. Biol.* **305**, 989–1010 (2001).
A thorough review describing different aspects of protein stability of antibody fragments and the successful engineering applications of antibodies to increase their stability.
59. Padlan, E. A. A possible procedure for reducing the immunogenicity of antibody variable domains while preserving their ligand binding properties. *Mol. Immunol.* **28**, 489–498 (1991).
60. Adair, F. Monoclonal antibodies: magic bullets or a shot in the dark. *Drug Disc. World* **3**, 53–59 (2002).

61. Ghetie, V. & Ward, E. S. Multiple roles for the major histocompatibility complex class I- related receptor FcRn. *Annu. Rev. Immunol.* **100**, 245–251 (2000).
62. Chapman, A. P. PEGylated antibodies and antibody fragments for improved therapy: a review. *Adv. Drug Deliv. Rev.* **54**, 531–545 (2002).
63. Anderson, W. L. & Tomasi T. B. Polymer modification of antibody to eliminate immune complex and Fc binding. *J. Immunol. Methods* **109**, 37–42 (1988).
64. Suzuki, T., Kanbara, N., Tomono, T, Hayashi N. & Shinohara, I. Physiochemical and biological properties of poly (ethylene glycol)-coupled immunoglobulin G. *Biochim. Biophys. Acta* **788**, 248–255 (1984).
65. Bona, C. A., Casares, S. & Brumeanu, T. D., Towards development of T cell vaccines. *Immunol. Today* **19**, 126 –133 (1998).
66. Lunde, E. *et al*. "Troy-bodies": recombinant antibodies that target T cell epitopes to antigen presenting cells. *Int. Rev. Immunol.* **20**, 647–673 (2001).
67. Lunde, E., Munthe, L. A., Vabo, A., Sandlie, I. & Bogen, B. Antibodies engineered with IgD specificity efficiently deliver integrated T-cell epitopes for antigen presentation by B cells. *Nature Biotechnol.* **17**, 670–675 (1999).
68. Rasmussen, I. B., Lunde, E., Michaelsen, T. E., Bogen, B. & Sandlie, I. The principle of delivery of T cell epitopes to antigen-presenting cells applied to peptides from influenza virus, ovalbumin, and hen egg lysozyme: implications for peptide vaccination. *Proc. Natl Acad. Sci. USA*. **98**, 10296–10301 (2001).
69. Dhodapkar, K. M., Krasovsky, J., Williamson, B. & Dhodapkar, M. V. Antitumor monoclonal antibodies enhance cross-presentation of cellular antigens and the generation of myeloma-specific killer T cells by dendritic cells. *J. Exp. Med*. **195**, 125–133 (2002).
70. Irving, R. A., Coia G., Roberts, A., Nuttall, S. D. & Hudson, P. J. Ribosome display and affinity maturation: from antibodies to single V-domains and steps towards cancer therapeutics. *J. Immunol. Methods.* **248**, 31–45 (2001).
71. Xu, L. *et al*. Directed evolution of high-affinity antibody mimics using mRNA display. *Chem. Biol.* **9**, 933–942 (2002).
72. Fitzgerald, K. *In vitro* display technologies- new tools for drug discovery. *Drug Discov.Today* **5**, 253–258 (2000).
73. Milstein, C. & Cuello, A. C. Hybrid hybridomas and their use in immunohistochemistry. *Nature* **305**, 537–540 (1983).
74. Glennie, M. J., McBride, H. M., Worth A. T. & Stevenson, G. T. Preparation and performance of bispecific F(ab' γ)2 antibody containing thioether-linked Fab' γ fragments. *J. Immunol.* **139**, 2367–2375 (1987).
75. Kettleborough, C. A., Saldanha, J., Heath VJ, Morrison, C. J. & Bendig, M. M. Humanization of a mouse monoclonal antibody by CDR-grafting: the importance of framework residues on loop conformation. *Protein Eng.* **4**, 773–783 (1991).
76. Saldanha, J. W., Martin, A. C. & Leger, O.J. A single backmutation in the human kIV framework of a previously unsuccessfully humanized antibody restores the binding activity and increases the secretion in cos cells. *Mol. Immunol.* **36**, 709–719 (1999).
77. de Wildt, R. M., Mundy, C. R., Gorick, B. D. & Tomlinson, I. M. Antibody arrays for high-throughput screening of antibody-antigen interactions. *Nature Biotechnol.* **18**, 989–994 (2000). **Describes a method for screening thousands of antibody-producing colonies. Further refinement and the use of this method will certainly be important in the antibody discovery field.**
78. Das, R. C. & Morrow, K. J. Jr. *Antibody Therapeutics: Production, Clinical Trials and Strategic Issues*. (D&MD Publications, 2001).

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