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Technological Evolution in the Development of Therapeutic Antibodies

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ABSTRACT

Immunotherapy is defined as the use of the immune system or components of it, such as key immune molecules, to fight diseases or invading infectious agents. Modern biotechnology provides industrial versions of immune molecules (components of the immune system) naturally produced by the human body. Immune molecules such as monoclonal antibodies are used as therapeutics in several disease conditions. In recent years a new group of antibody based molecules has been developed to replace monoclonal antibodies, given their ability to overcome some of the limitations of the latter. The first clinical trials with these new molecules have been very encouraging and the promise is that they will be released to the market very soon. This in turn has stimulated more research on new versions of antibody based therapeutics by biotechnological companies supported by the pharmaceutical industry and in many cases in collaboration with academic institutions. (REV INVES CLIN. 2015;67:158-69)
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THE CHALLENGE OF CANCER THERAPY

After more than half a century of research in chemotherapy, cancer remains one of the most difficult diseases to cure. This is a consequence of factors including cellular diversity, tumor biochemical heterogeneity, drug resistance and adverse effects, and the limitations of studying new anticancer drugs in animal models. The incidence of cancer is increasing due to environmental and lifestyle factors that are just being understood.

Throughout the world, changes in diet, physical activity, and average body weight have occurred as countries become more industrialized, with these changes accounting for as much as 30% of all cancers. It is estimated that in 20 years, new cancer cases will increase from approximately eight million to more than 20 million worldwide each year[1,2].

The goal of cancer therapy is to eliminate malignant cells without damaging healthy cells of the patient. Along with chemotherapy, current treatments for this

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disease are mainly based on surgery and radiation, which are effective locally and limit damage to the healthy cells in tissues near the tumor. The problem with these therapeutic options is that although they do not cause damage to normal cells distant from the tumor, they also do not affect malignant cells that have moved to other locations (metastasis). Chemotherapy, however, penetrates the entire body and can eliminate malignant cells that are beyond the local tumor area, but unfortunately it is not selective enough and therefore also damages normal tissues. Although certain cancer drugs may have some specificity to inhibit a particular enzyme or damage certain protein structures, these targets are still found in normal tissues, hence the toxicity of these drugs[3].

The "selectivity" of chemotherapy is generally attributed to the increased susceptibility of tumor cells over normal cells to chemotherapeutic agents. Furthermore, there is the excessive biochemical stress to which malignant cells are subjected due to the increase in cell proliferation, demand for energy, and time to repair damage and errors in the genetic material before entering the next cell division of the actively dividing cells characteristic of tumors. Although this strategy is somewhat effective, there is also considerable damage to normal cells undergoing proliferation. This damage is seen as the toxicity of therapy, sometimes producing a painful and undesirable result, which limits its use. If cancer treatment could be directed and released specifically in tumor cells, there would be a marked reduction in the toxicity associated with therapy. If so, therapy could be administered safely and possibly at greater doses, which would result in its being more effective[4].

IMMUNOTHERAPY

In the last decade of the 19th century, the German army doctor Emil von Behring used blood serum from horses for the treatment of tetanus and diphtheria (blutserumtherapie, the German word for serum therapy). When the data were published in 1890 (Behring and Kitasato, 1890), very little was known about the factors or mechanisms involved in immune defense. Despite this, the conclusion was that the human body needs some defense mechanism to fight against external infections and their molecules, and the enabling molecules must be present in the blood (and therefore, the serum can be prepared and used as therapy against the toxins in infections)[5].

In 1895, Hericourt and Richet reported the first clinical study that analyzed the principle of antibody production. They injected tumor cells in animals to obtain antisera to treat cancer patients; some patients showed improvement, but none could be completely cured[6].

The German physician Paul Ehrlich was the first to propose, in the early 20th century, the term "magic bullets". A Nobel Prize winner in 1908, Ehrlich described the use of antisera and antibodies in the treatment of diseases employing cell-based vaccines or antigens, which he called "passive immunization". According to his theory, the antibodies could work as magic bullets to kill only tumor cells without affecting normal cells. Between 1925 and 1980 numerous clinical studies were conducted in this field, which were limited by the small quantities of purified antitumor antisera obtained, the lack of purity of the preparations, and the difficulty to produce new lots of antisera[7].

One of the first reports of the use of antisera in cancer treatment was by Lindstrom in 1927, who tried 15 administrations of rabbit antiserum in 10 patients diagnosed with chronic myeloid leukemia[8]. He noted a decrease in peripheral blood myeloid cells in five of the 10 cases, but highly significant side effects were attributed mainly to impurities of the preparation. Unfortunately, the success of immunotherapy against cancer was not as expected, since the obstacles mentioned earlier limited the enthusiasm for this innovative immunotherapy against cancer[9].

A NEW TECHNOLOGY, A NEW HOPE FOR IMMUNOTHERAPY

A new hope to explore the area of immunotherapy arrived in 1970, when it was found that myeloma cells (B-cells) produced a single type of antibody. In 1975, a revolution in the generation of antibodies was started when Hans Kohler and Caesar Milstein published the successful fusion of this class of myeloma cells with splenocytes immunized with red cells. The fusion resulted in the infinite production of cell lines that synthesize a single type of antibody; the method was called "hybridoma technology". For this work, these

researchers were awarded with the Nobel Prize in 1986[10]. Following the publication of this article, numerous academic laboratories and emerging biotechnology companies began to develop monoclonal antibodies (mAb) by immunizing mice with human cells and antigens.

To produce mAbs by hybridoma technology, mice (or other animals) are immunized with the antigen of interest. After 72 hours from the last booster, the spleen is removed and a suspension of splenocytes is prepared; the splenocytes are then mixed with mouse myeloma cells growing in suspension. A chemical reagent or a virus is added to promote fusion of the membranes of both cell lines. This leaves the formation of random cell fusions (splenocytes-myeloma cells, splenocytes-splenocytes and myeloma cells-myeloma cells). Selection is performed by culturing the newly fused hybridoma cells in a special medium containing hypoxanthine-aminopterin-thymidine (HAT medium) for 10-14 days. Aminopterin blocks the *de novo* pathway of purine biosynthesis, which kills un-fused myeloma cells since these cells are unable to produce nucleotides by the *de novo* pathway or the classical pathway. Under these conditions, only the splenocyte-myeloma cell hybrids can survive and propagate[11]. Hybridomas are capable of secreting single specificity antibodies (mAbs), therefore overcoming the difficulties of "antitumor sera."

FIRST CLINICAL USE OF MONOCLONAL ANTIBODIES

The first clinically available mAb was muromonab-CD3 (Orthoclone OKT3), a murine antibody used to reverse acute renal graft rejection and approved by the US Food and Drug Administration (FDA) in 1986. After eight years, in 1994, abciximab (ReoPro®), the first chimeric antibody composed of variable regions of a mouse antibody and the constant region of a human antibody was approved for cardiovascular use as an inhibitor of platelet aggregation. In 1997, the first antitumor mAb, rituximab (Rituxan®), as well as daclizumab (Zenapax®), for the prevention of kidney transplant rejection, were approved[12]. From then on, mAbs have become a group of products with a high clinical and economic impact. To date, 26 mAbs have been approved by the FDA for various clinical applications, including neoplastic, infectious, cardiovascular, and autoimmune diseases. This class of therapeutic antibodies constituted a 32-billion dollar market in 2008, estimating that 30% of biologic drugs correspond to mAbs and anticipating that in the future, the figure will be 9% of the total pharmaceutical market[13,14].

NOMENCLATURE OF MONOCLONAL ANTIBODIES

With the rise of mAbs and the large number of these that are under development, a nomenclature system has become necessary to provide information about their origin and uses. In 2008, a working group meeting of the International Nonproprietary Name Program (INN) was convened by the World Health Organization to review and establish the guidelines of the nomenclature system for mAbs[15]. In this meeting, the following naming system for mAbs was approved:

– **Prefix:** The prefix should be random, e.g. the only requirement is to contribute to a euphonious and distinctive name.

– **Substem A:** Indicates the target (molecule, cell, organ) class. In principle, a single letter is used, e.g. *-b-* for bacterial, *-t-* for tumor. Whenever substem B starts with a consonant (e.g. x or y), to avoid problems in pronunciation, an additional vowel is inserted, e.g. *-ba-*, *-tu-*.

– **Substem B:** Indicates the species on which the immunoglobulin sequence of the mAb is based.

– **Suffix:** *-mab*; all the products that contain an immunoglobulin variable domain that binds to a defined target.

Prefix, Substem A, Substem B, suffix

Examples:

– Ri/tu/xi/mab: anti-lymphoma monoclonal chimeric.

– Ab/ci/xi/mab: chimeric monoclonal that prevents coronary thrombi.

– Ada/li/mu/mab: human monoclonal for rheumatoid arthritis.

THERAPEUTIC MONOCLONAL ANTIBODIES

Currently, there are over 200 mAbs in clinical studies and the number entering clinical trials is increasing each year. The success of mAbs is most evident for anticancer agents where they have led to major advances in treating common malignancies, such as breast cancer (e.g. trastuzumab), colorectal cancer (e.g. cetuximab), lymphoma (e.g. rituximab) and leukemia (e.g. alemtuzumab). The majority of mAbs both approved and in clinical trials are primarily intended for oncology indications, such as:

Rituximab

Rituximab (Rituxan®, MabThera®) is a chimeric mAb targeting CD20 antigen, expressed by B-lymphocytes, from the pre-B to the mature germinal center B-cells, and by most B-cell neoplasms derived from these cells[16-18]. Approved by the FDA since 1997, this molecule acts by antibody-dependent cell-mediated cytotoxicity (ADCC), cytotoxicity mediated by complement and lately discovered to activate a signaling pathway of cell death. Rituximab is used in combination with polychemotherapy in the treatment of all histological types of B non-Hodgkin lymphoma (B-NHL) and in chronic lymphocytic leukemia, both as first-line and as rescue therapy. Furthermore, it is used for maintenance therapy of B-NHL and for the treatment of several autoimmune diseases, in particular rheumatoid arthritis[19-21].

Trastuzumab

Human epidermal growth factor receptor 2 (HER2) is a transmembrane tyrosine kinase receptor overexpressed in 25-30% of breast cancers. In 1998, anti-HER2 trastuzumab (Herceptin®, Roche) became the first humanized mAb to obtain FDA approval. Trastuzumab as a single agent is indicated for the treatment of patients with metastatic breast cancer whose tumors overexpress the HER2 protein and who have received one or more chemotherapy regimen. Trastuzumab is also approved for use in combination with paclitaxel for the treatment of patients with HER2-expressing metastatic breast cancer who have not received chemotherapy for their metastatic disease[22,23].

Cetuximab

The epidermal growth factor receptor (EGFR) is a 170 KDa membrane protein, and its aberrant expression or activity has been identified as a key player in many human epithelial cancers, including head and neck squamous cell carcinoma (HNSCC), non-small cell lung cancer (NSCLC), colorectal cancer (CRC), breast, pancreatic, and brain cancer[24]. The EGFR is a member of the EGF tyrosine kinase receptor family, which consists of the EGFR (ErbB1/HER1), HER2/neu (ErbB2), HER3 (ErbB3) and HER4 (ErbB4)[25]. Cetuximab (Erbitux®) is a human/murine chimeric mAb that binds to the extracellular domain III of EGFR; this interaction partially blocks the ligand-binding domain and sterically hinders the correct extended conformation of the dimerization arm on domain II. Thus, cetuximab prevents both ligand binding and the proper exposure of the EGFR dimerization domain, preventing activation of the EGFR pathway[26-28]. Cetuximab has shown antitumor activity in the clinical setting as either monotherapy or in combination with chemotherapy and/or radiation, particularly in metastatic CRC (mCRC) and HNSCC. In 2004, the FDA approved cetuximab for use in patients with EGFR-expressing mCRC refractory to irinotecan-based chemotherapy. Since this approval, several extensive clinical trials have supported the use of cetuximab in mCRC[29-38].

MECHANISMS OF ACTION OF THERAPEUTIC MONOCLONAL ANTIBODIES

Therapeutic mAbs work through several mechanisms that have been divided into two classes: (i) those in which the effect is carried out independently of the body's immune system, and (ii) those that require the participation of an immune response system[39].

Immune system-independent mechanisms

Action on a signaling pathway

In vitro studies have shown that some mAbs induce cell death by binding to a receptor protein, triggering a signaling cascade that results in cell death or growth inhibition[40].

Disruption of the interaction between a ligand and its receptor

Monoclonal antibodies can block the activation signals necessary for the development and maintenance of tumor cells. This is done by removing the ligand from the circulation, blocking the interaction between the ligand and its receptor, inducing receptor modulation, or by interfering with ligand-induced dimerization of the receptor[41].

Immune system-dependent mechanisms

Cytotoxicity mediated by complement

This mechanism takes place after the binding of antibodies to the target cell and the union of the free Fc portion of antibodies with proteins of the complement system, which leaves, as a result, membrane rupture and cell destruction. For this type of cytotoxic effect to occur, the antibody must be whole or include the Fc portion, plus any other subunit of the antibody that binds to the antigen (Fv or Fab). Among the factors that have been studied and that affect the clinical response to therapeutic antibodies that base their cytotoxicity on this mechanism are polymorphisms in the gene of the C1qA [276A/G] complement component, which affect the response and duration of therapy. For example, this is the case of rituximab treatment for follicular lymphoma. It is known that homozygosity for the 276G allele correlates with a higher serum concentration of C1q protein, which shortens the progression time compared with the 276A allele[42].

Antibody mediated cell cytotoxicity

This cytotoxic mechanism operates with the aid of immune effector cells. Once the variable region of the antibody binds to its target antigen, the Fc portion binds to its receptor (FcγR) on the cell surface of an immune effector cell (NK cells, monocytes, macrophages, etc.), which also triggers recruitment of adapter proteins and activation of immune response effector cells[43]. In this type of mechanism, a crucial factor for the effectiveness of cell cytotoxicity of the antibody falls on the number of receptors and their affinity for the Fc portion of the antibody, which predicts the degree of antibody mediated cytotoxic activity (ADCC). Furthermore, in animal models it has been found that there is a depletion of effector cells, consequently reducing the effectiveness of the antibody. Fc receptor on macrophages and NK cells (FcγRIIIa) has two isoforms polymorphic at residue 158, which specify either valine or phenylalanine. Of these isoforms, FcγRIIIa-Val158 has a higher affinity for the Fc portion of antibody than FcγRIIIa-Phe158, which increases the activity of effector cells[44].

ANTIBODY ENGINEERING

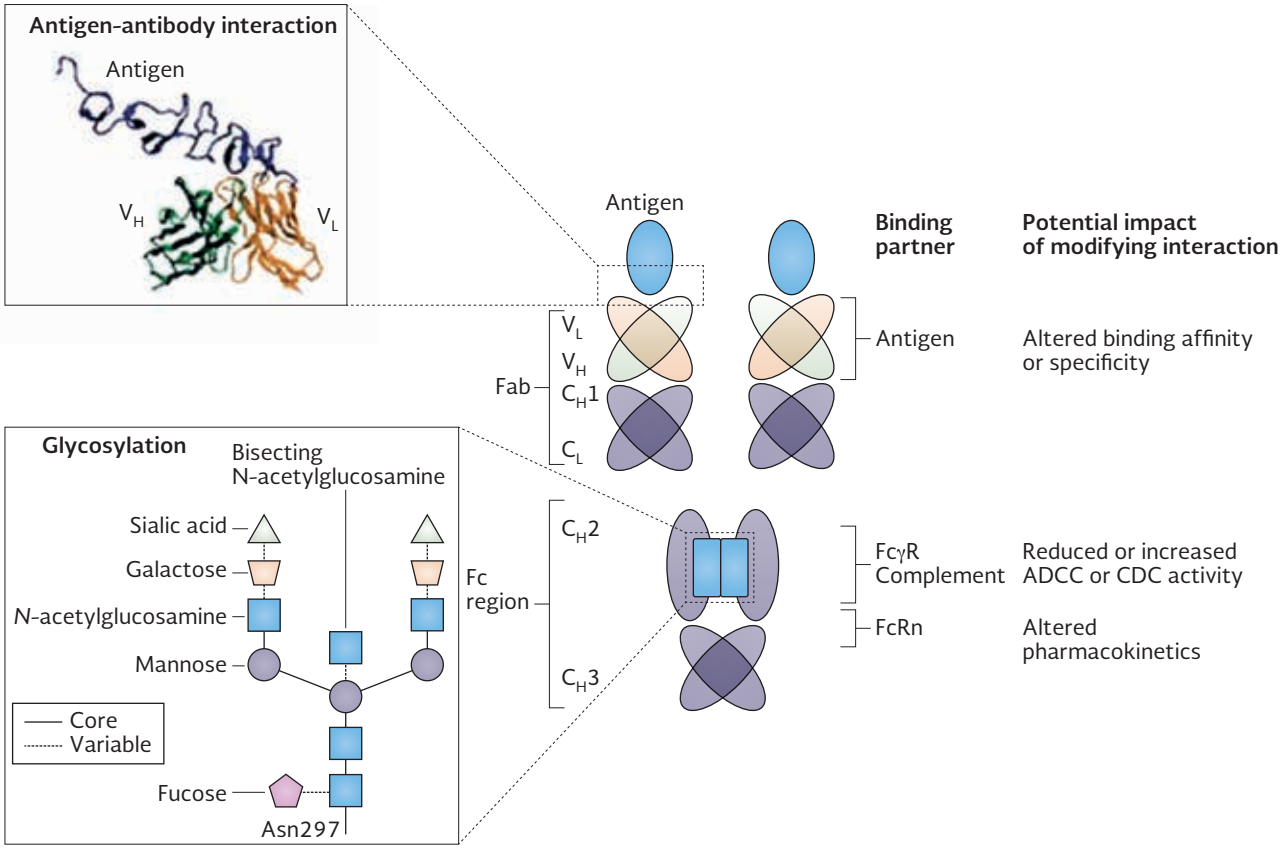
In general, antibodies are polypeptide chains with a tetrameric configuration made up of two heavy chains and two light chains. Each light chain is linked to the heavy chain by a disulfide bond, and the heavy chains are connected by two disulfide bonds (Fig. 1). An antibody can be fragmented by enzymatic digestion, leaving as a result structures called Fab, F (ab')2 and Fc. Furthermore, each light chain has a variable (Fv) and a constant (Fc) region, while each heavy chain has a variable region and three to four constant regions, depending on the class of immunoglobulin (Ig)[9,45,46]. The antibody effector function is mediated by the constant domains of both heavy chains (Fc)[47]. The diversity of the immune response is possible due to several combinations and permutations of regions coding for heavy and light chain regions referred to as variable (V), diversity (D) and joining (J), which combine to generate heavy chains (V, D, and J) and others that combine to produce light chains (V and J)[48].

The antigen-binding domain is composed of variable regions of one light and one heavy chain, so each antibody can bind to two copies of its target antigen. The basis for the specificity of antibodies rests on the variable regions of both chains (Fv), specifically in the sequence of amino acids that make up the hypervariable sub-region (Fhv). This region is the "idiotype" of Ig. The six hypervariable sub-regions of the variable regions may also be referred to as complementarity determining regions (CDR)[49].

Problems of monoclonal antibodies

The simplicity of the technique of immunizing a mouse with any antigen promised antibody production for almost anything. However, when the mAbs obtained from mouse cells were employed clinically, their use was found to be limited. Murine antibodies

Figure 1. Antibody structure (immunoglobulins). Domains are formed in the antibody because of the link between heavy chains and light chains through disulfide bonds, and 3D structure of the antigen-antibody binding domain interaction, responsible for antigen recognition[50]. ADCC: antibody-dependent cell-mediated cytotoxicity; CDC: complement dependent cytotoxicity. *Reprinted with permission from Macmillan Publishers Ltd: Nat Rev Immunol[50], copyright 2006.*



were rapidly inactivated by human antibodies produced against them. This type of immune response is called human anti-mouse antibodies (HAMA), and it not only causes flu-like symptoms, an allergic reaction, and in extreme cases, shock and death, but also the rapid inactivation and elimination of the murine mAbs after administration[51].

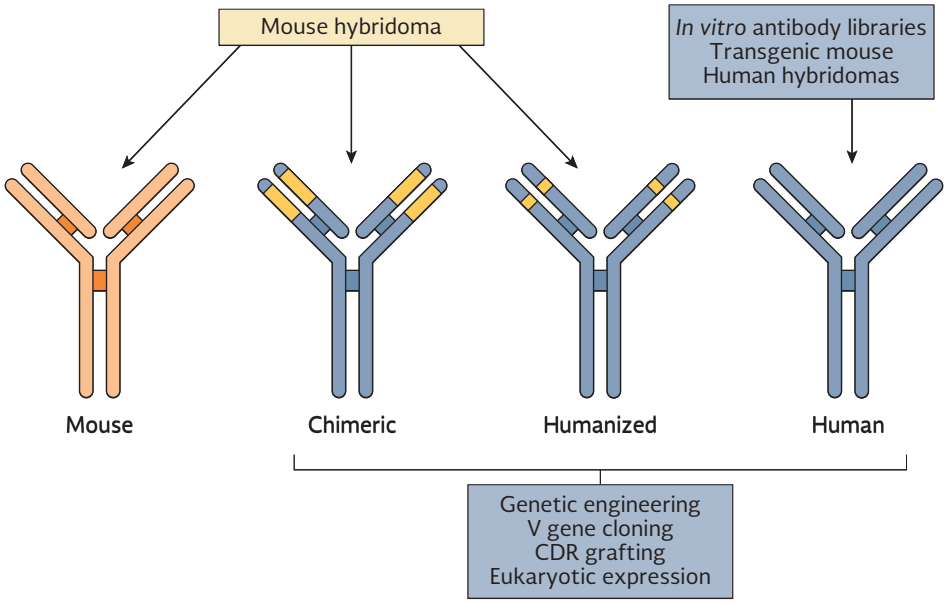
Chimeric, humanized and human antibodies

To solve the problems associated with the HAMA response, a research area called "antibody engineering" emerged, which allowed the development of chimeric antibodies. The antigen binding ability of the antibodies is generated by the Fv portions of the heavy and light chains, while the Fc portion is responsible for mediating the binding to effector cells and the subsequent immune response. This made it possible to create chimeras in which the constant region of the mouse antibody was replaced by the human constant region, resulting in an antibody having the binding characteristics of a mouse antibody and the ability to translate signals from the human antibody and/or trigger response mechanisms with the action of the immune system. These chimeric antibodies are 30% mouse and 70% human so they are likely to generate a HAMA response. In an attempt to reduce further the HAMA response of murine and chimeric antibodies, in 1986, Winter, et al. produced a human antibody in which they inserted mouse CDRs using a technique called "complementarity-determining region graft." These antibodies are more human (90-95%) than chimeric and are called "humanized antibodies" (Fig. 2)[10].

Phage display technique

With improved techniques of genetic engineering, it was possible to clone within phage genomes the

Figure 2. Comparison of different types of monoclonal antibodies. Genetic engineering technologies have allowed the replacement of murine fractions of antibodies for human fractions, reducing the human anti-mouse antibody response[58]. CDR: complementarity determining region. *Reprinted with permission from Macmillan Publishers Ltd: Nat Rev Drug Discov[58], copyright 2003.*



entire gene base that forms part of the battery of antibodies of animal and even human cells, immunized and unimmunized. This allowed the reproduction on the surface of bacteriophages, of more than 1 x 1,011 antigen-binding sites, with the specificity of an antigen per phage particle. The phage carries in its genome the coding sequence for the antibody of interest, and this technique results in the expression of the antibody on the phage surface, which allows for the *in vitro* selection by affinity chromatography using the antigen of interest as the ligand[52].

Affinity maturation of antibodies

Another option for developing a new class of antibodies is by generating large numbers of variants containing mutations in controllable positions in the antibody sequence, specifically in the CDR portion[50]. There are several strategies for mutagenesis available, including random approaches such as error-prone PCR or DNA shuffling[53,54]. Variants with improved binding kinetics can be isolated by tailoring the selection conditions such that they are preferentially enriched, for example, by lowering the concentration of the antigen or increasing the time of incubation with the antigen[55,56]. These approaches have led to many examples of *in vitro* matured antibodies with affinities beyond those naturally found in the immune response, with some examples exhibiting equilibrium dissociation constants in the femtomolar range[57] (Fig. 2).

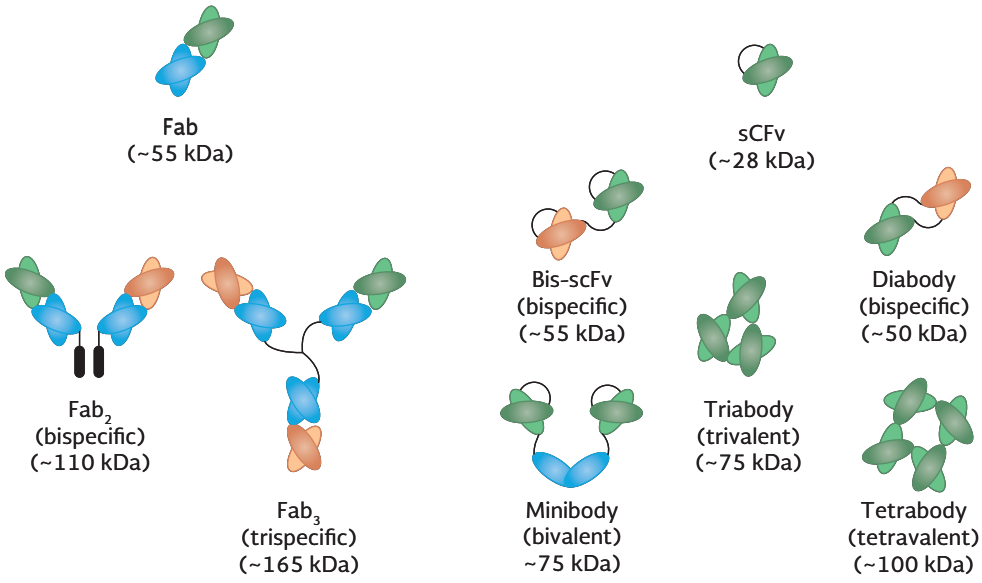
Transgenic mice

Another tool for generating fully human antibodies is the "transgenic mouse". In this case, the mouse is created by exchanging its repertoire of IgG genes for that of human IgG. After immunization, the mouse produces human antibodies against the antigen used for immunization. Subsequently, using hybridoma technology, the clone that meets the appropriate requirements in terms of antigen specificity and quantity can be obtained[59].

FRAGMENTS OF ANTIBODIES

Humanized mAbs are generally well tolerated and many have a very favorable clinical response from patients. Such is the case of rituximab (Anti-CD20) in hematological tumors. Unfortunately, none of the antibodies developed so far can be used as monotherapy for the treatment of any disease. Different clinical studies and studies in animal models have shown evidence of the limitations of these "magic

Figure 3. Representation of different types of antibody fragments. Outline of the formats that are being developed to enhance the activity of conventional monoclonal antibodies[78]. *Reprinted with permission from Macmillan Publishers Ltd: Nat Biotechnol[78], copyright 2005.*



bullets", among which are: their mode of action (redundancy in the mechanisms of action that leave behind the survival of tumor cells), limited penetration because of their large size, half-life in serum of the molecules capable of exerting an effect, the effects of the microenvironment, factors affecting the affinity of the Fc portion (glycosylation) for its receptor (polymorphisms), activation of inhibitory receptor isoforms (FcγRIIa), and competition with circulating IgG, among others[60].

Many of these limitations have been overcome with new genetic engineering techniques that have allowed the development of the antibodies dreamed of 25 years ago: "magic bullets" that are not found in nature, but are based on a natural model of human immunoglobulins. These proteins have the ability to redirect effector cells, which express the receptor Fcγ, towards the cell expressing the target antigen and that trigger antibody dependent cytotoxicity (ADCC)[61]. This ability to recognize two surface proteins of different cell populations has been exploited for the development of bispecific antibodies (BsAbs). Thus, these antibodies, which are strictly antibody fragments, are designed to have the ability to join two targets simultaneously, i.e. redirect effector cells (B/T lymphocytes, monocytes, macrophages, NK cells, etc.) to target cells (those of tumors, for example). The engineering of proteins (antibodies) has generated formats such as diabodies in tandem, quadromas, Fab2 and single chain antibodies (Fig. 3)[62].

The first BsAb was developed much like a normal IgG, but it had in its two Fv regions a binding capacity to two different antigens. The potential use of these BsAbs to redirect an effector cell to a tumor cell was demonstrated during 1980; from there, several phase I clinical trials were conducted in the early 1990s. The first BsAbs that reached phase I clinical trials were constructed via two antigen-binding fractions (Fab)2 chemically bonded, which redirect CD64 positive effector cells (monocytes and macrophages) toward tumor cells that express antigens such as HER2 or EGFR[63,64]. But these BsAbs did not have the expected clinical effect because the antitumor activity was very inconsistent, partly because of the need for a high concentration of the antibody to achieve the cellular dependent cytotoxicity, and partly due to the need for a 40:1 (E:T) ratio between effector cell and target cells, even in patients pre-stimulated with interferon-γ and granulite colony stimulating factor.

A different approach, but under the same concept of BsAbs, was the re-routing of more abundant and potent killer cells from the immune system, T-cells. Because of their ability to proliferate after activation, to

kill at different times, to infiltrate tumors, and the lack of FcγR receptor binding to antibodies, it was thought that the binding of an antibody to a target antigen (tumor) and an antibody directed to a T lymphocyte receptor (CD3), would result in the re-directing of the T-cell response. As with the BsAb that use CD64 to attract effector cells, these antibodies promise much more than expected. The need for T lymphocyte activation by the CD28 receptor and its ligand B7 (which are essential for T-cell activation) was the problem with these versions of antibodies, so the first BsAb-CD3 was administered with an anti-CD28, but this coadministration yielded inconsistent results[65].

The solution to this problem of co-activation came with the development of antibody engineering in the 1990s, where a single chain variable fragment (scFv) was developed by fusing the variable domains of heavy and light chains through a peptide linker. This allowed the tandem fusion with another scFv through an additional connector in such a way that four peptides are aligned in a single chain of about 55 kDa, as opposed to initial BsAbs in which the variable fragments belong to two different polypeptide chains[25,66-73].

The most successful type of antibody to date was developed by the company Amgen (Thousand Oaks, CA, USA). This bispecific antibody is a T-cell carrier (BiTE®), and it is designed by fusing the scFv taken from an anti-CD19 with the scFV from an anti-CD3 in a single polypeptide chain (scFv) through a five amino acid connector, with the ability to redirect T-cells and cause lysis without needing co-stimulation[74-77] (Fig. 3).

UNUSUAL ANTIBODIES

As mentioned before, antibodies are composed of two heavy chains linked to two light chains by two disulfide bonds. In addition to these conventional antibodies, camelids and sharks produce some unusual antibodies with only heavy chains in their structure. This particular heavy chain antibody lacks light chains (in the case of camelids, the CH1 domain). The variable domain (Fv) of these organisms is designated as VHH for camelid antibodies and VNAR for shark antibodies, although they can be designated both as single domain antibodies[79-84]. Due to the lack of a light chain in these antibodies, changes in antigen-binding domains are expected to fill the absence of light peptides. To fill these gaps, the CDR3 of VHHs and VNARs is longer than the conventional VH sequence, about 24 and 18 amino acid residues, respectively, compared with the seven of conventional VH. This longer CDR3 is stabilized by the addition of a cysteine residue, which creates a disulfide bond with one of the other CDRs[85]. It was also found that once cloned and isolated, these VHHs and VNARs do not lose their antigen-binding ability, making them the basis for the development of Nanobodies® by the biotech company Ablynx[86,87].

These nanobodies have several advantages over the conventional antibodies, such as good specificity and affinity for their target, while behaving as small-molecule drugs because they can, for example, inhibit enzymes by binding to their active sites and gain access to receptors that a conventional antibody would not[88].

FROM LAB TO MARKET

After more than 100 years of research in immunotherapy, no significant advances were seen until the discovery of mAbs. Today, innovation is coming back to immunology because of the results obtained with fragments derived from antibodies that are capable of binding and neutralization.

The advantage of antibody fragments falls in not requiring huge investment costs as is required in molecular modeling; this is because of the need for supercomputers that can carry out the *in silico* analysis. Another advantage is the presence of multiple biotech companies that have become large strategic partners for the development of these antibody fragments.

The results of phase II clinical trials of BiTE® (blinatumomab) have excited the pharmaceutical companies, which have started research on new formats with similar results to develop their own molecules (Table 1).

Amgen, based in the USA, is a leading biotechnology company that has invested in developing these new formats arising from antibodies, and whose main

Table 1. Biotech companies that are currently developing antibody derived fragments

Company	Technology platform	Mechanism
Macrogenics	DART® (dual-affinity re-targeting) platform	Dual specificity 'antibody-like' therapeutic proteins capable of targeting many different epitopes with a single recombinant molecule.
f-star	Modular antibody technology	Allows small antibody fragments with full antibody functionality (Fcab) or full-length antibodies with additional functionality (mAb) to be created.
Amgen	BiTE® (bispecific T-cell engager) technology	Lead product blinatumomab (MT-103) is a BiTE® antibody designed to direct T-cells against CD19 on B-cell-derived acute lymphoblastic leukemia and non-Hodgkin's lymphomas.
Bicycle Therapeutics	Bicycle technology	Mini-antibodies with two binding loops covalently attached to organochemical cores.
Domantis/GSK	Dual targeting dAbs	The fully human dual targeting dAbs bind two targets simultaneously and can be manufactured in dimer, Fab-like or IgG-like formats. Domantis is developing dual targeting factors for solid tumors tumor antigens on tumor cells and angiogenic dAbs against cytokines for inflammatory diseases, tumor antigens on tumor cells and angiogenic factors for solid tumors.

dAb: domain antibody; Ig: immunoglobulin.

molecule (BiTE®) has attracted the attention of partners from the pharmaceutical industry, such as Bayer-Schering, Sanofi, and Boehringer Ingelheim, which have invested in the development of these derived antibodies based on the BiTE® format[61].

On the other hand, Macrogenics, the leading competitor of Amgen, has attracted the interest of pharmaceutical companies for the development of their main antibody derived molecule, which is a bispecific molecule called DART® (Dual Affinity Re-Targeting)[76].

Another company that is developing a different format is Ablynx, a company from Belgium of no more than 100 workers, whose main focus is the use of single-chain antibodies isolated from camelids and sharks (Nanobodies®). Ablynx has been able to attract several partners who have invested large sums of money for the development of Nanobodies®[86].

CONCLUSIONS

The examples shown above illustrate the role that antibody derived molecules will play in the pharmaceutical industry in the coming years, and according to the results from their clinical trials, they seem very promising as therapeutics. This is why many research groups are betting on the development of these new types of molecules that are better at directing the immune response of our effector cells against tumoral cells.

DECLARATION OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

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