REVIEW

Therapeutic Monoclonal Antibodies

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Over a hundred years has passed since the discovery of the "magic bullet" serum therapy by Kitasato and Behring, the first ever therapeutic use of antibodies. More than 80 years later, the investigation of immunoglobulin structure and function and the development of cell and molecular biology introduced the production of monoclonal antibodies (MoAbs). In the 35 years since the first process for creating MoAbs was introduced, they have remained the centerpiece of the growing biotechnology and pharmaceutical industry. Herein, I review the history, development, and clinical settings of therapeutic MoAbs that have had a significant impact on life-saving medicine. (Keio J Med 60 (2) : 37–46, June 2011)

***Keywords*: monoclonal antibody, molecular target therapy, immunoglobulin**

Introduction

Shibasaburo Kitasato and Emil von Behring showed that the serum from human patients or horses who had recovered from an infectious disease could be used to prevent and/or treat the disease in other people or animals. Human immunoglobulins (Igs) obtained from donors are used to treat a variety of infectious diseases; however, these applications are restricted by availability and limited potency. Monoclonal antibodies (MoAbs) resolved many limitations in the clinical usage of Igs. The first MoAb for clinical use, OKT3 against T lymphocytes, was generated as a therapy against tissue rejection. Initially, the field of MoAb therapy developed slowly because of the time required to improve methods for humanizing murine MoAbs and to develop manufacturing processes and capability. Some novel procedures for humanizing murine MoAbs decrease the antigenicity of Igs and provide progressively greater potency of MoAb therapy. This article provides a comprehensive review of the development, characterization, and clinical application of therapeutic antibodies.

Origin of Serum Therapy

In the year 1882, Robert Koch, the discoverer of the tubercle bacillus, assembled a team to tackle the newly forming field of immunotherapeutics; the team included Paul Ehrlich, Emil von Behring, Erich Wernicke, and Shibasaburo Kitasato, all of whom would have a significant impact on the beginnings of antibody-based therapy. Behring made some key observations that led to the concept of serum therapy. He noticed that the blood of those rats resistant to anthrax was able to kill the anthrax bacterium. Together with Wernicke, he developed the first working serum therapy for diphtheria. Kitasato and Behring had isolated the tetanus-forming bacillus and had determined that its pathogenesis lay in the activity of a toxin, and then demonstrated that the transfer of serum from a guinea pig immunized with diphtheria toxin to another guinea pig offered protection from the toxin.[1] Kitasato and Behring also obtained anti-sera against tetanus toxin, demonstrating the breadth of the principle (**Table 1**). Their serum therapy against diphtheria was first tested clinically in 1891 at Charite Hospital in Berlin. A year later, Behring started working with the pharmaceutical manufacturer Farbwerke Hoechst to develop the diphtheria serum treatment. In 1894, Hoechst launched the first immunobiological therapeutic, dispatching 25,000 doses of anti-diphtheria serum to prevent epidemic diphtheria that took the lives of 50,000 children annually in Germany. The introduction of serum therapy decreased mortal-

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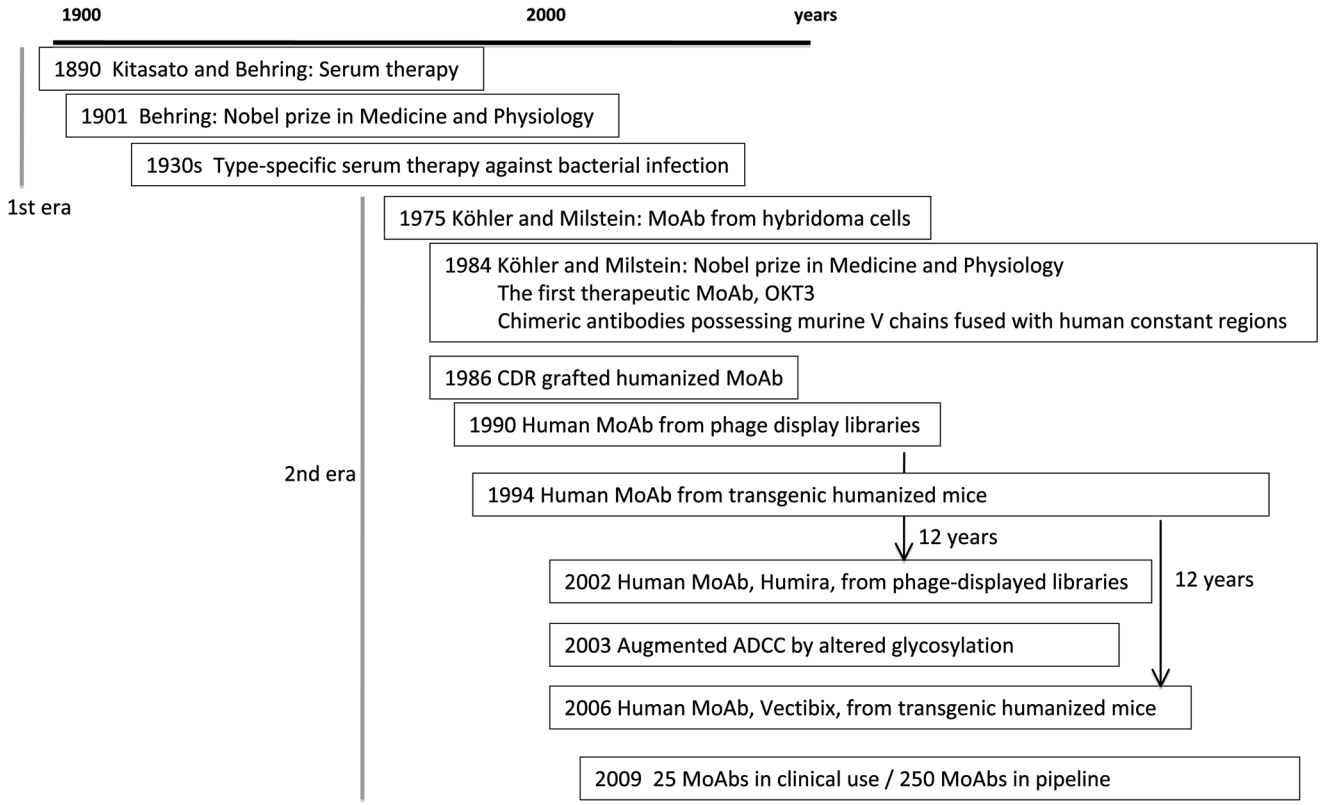
Table 1 The development of therapeutic monoclonal antibodies



There are two major eras in the development of therapeutic antibodies: 1) the discovery of serum therapy (1890~) and 2) the development of monoclonal antibodies (1975~). Therapeutic MoAbs are often developed 10-12 years after the establishment of novel technology.

ity in Paris from 52% to 25%. This success popularized the term "magic bullet," which was an apt and prescient description for present immunotherapy. The discovery of serum therapy led to the awarding of the Nobel Prize in Medicine and Physiology to Behring in 1901 (**Table 1**). Behring used the funds from this Nobel Prize to set up a new company in 1904, which today is known as Novartis (Chiron) Behring. It was likely the first example of venture capital funding in biotechnology. The serum for serum therapy was crude and came from immunized, non-human sources (horses or rabbits). It contained many foreign proteins and gave rise to a phenomenon that has been generally called "serum sickness." The onset of serum sickness in 100% of patients treated with serum therapy led to various efforts to improve on this approach. Behring recognized these toxic side effects of serum therapy and introduced improved methods for purification of serum.

Meanwhile, Ehrlich, another member of the team,realized the standardization of serum therapy, which led to the development of procedures for quantification of the serum therapeutic effect, including the concept of LD50. This serum therapy was used widely until approximately the onset of World War II for various bacterial diseases including diphtheria, meningitis, and pneumonia. An example of how widespread serum therapy had become is that 86% of patients diagnosed with type I streptococcal pneumonia in the late 1930s at Boston City Hospital were treated with a type-specific serum therapy (**Table 1**).[2] The second trial of serum therapy was done by isolating natural antibodies in either vaccinated or naïve humans, followed by isolation of IgG fractions from pooled serum for the therapy of a patient with primary immunodeficiency.[3]

Development of Monoclonal Antibodies (MoAbs)

Köhler and Milstein developed methods for the isolation of MoAbs from hybridoma cells in 1975 (**Table 1**).[4] They showed that the cell fusion technique could be used to produce hybrids between myeloma cells and antibody-producing cells. The resulting hybrid lines were permanently adapted to grow in tissue culture and were capable of inducing antibody production in mice using Jerne's hemolytic plaque assay, which allows direct visualization of antibody-producing B cells.[5] This discovery led to the

awarding of the Nobel Prize in Physiology or Medicine in 1984 to Köhler, Milstein, and Jerne. Köhler and Milstein did not patent this method, which opened up the use of their hybridoma technology to academics and pharmaceutical fields alike for generations of future potential therapy. The first therapeutic MoAb was generated as OKT3 by Ortho Biotech in 1984 (**Table 1**). The first step was the ability to clone out, using the PCR technique, murine VH and VL genes for recombinant expression.[6] The second step was to express both heavy and light chain antibody genes in stable human cell lines after transfection.[7] The third step was the method of producing chimeric antibodies possessing murine VH and VL chains fused with human constant regions.[8,9] Chimeric antibodies have about one-third murine sequences (2 VH and 2 VL subunits) and two-thirds human sequences, including a human Fc. For MoAb expressions, the pSV2 vector and SP2/0 murine myeloma cell line were widely used in the early period.[10] Recently, Chinese hamster ovary (CHO) cell lines and a glutamine synthetase (GS) expression system were used, as widely licensed by Lonza.[11] After cell cloning and optimization using this CHO-GS expression system, MoAb production is 1–5 g/L. Chimeric antibodies still retain one-third murine sequences, which may lead to enhanced immunogenicity.[12]

Humanization is the idea of making the V chains from a murine or other mammalian antibody "more human." The complementarity-determining regions (CDRs) were grafted from a murine antibody into the closely related human framework, followed by making amino acid changes required to stabilize the engineered constructs.[13] Queen et al. developed a detailed process for humanizing antibodies via CDR grafting, which was the basis for humanization of many MoAbs currently on the market or undergoing clinical trials.[14] Other procedures of humanization without CDR grafting have also been developed, most notably the resurfacing of antibodies to remove B cell epitopes.[15] The first humanized MoAb used as a therapeutic agent was an anti-CD25 (IL-2 alpha subunit) MoAb that was humanized and developed at Protein Design Labs and licensed by Roche to suppress rejection after transplantation. In contrast to these in vitro manipulations for humanizing MoAb, two research groups separately and independently were successful in developing functional human MoAbs directly from "humanized" transgenic mice (**Table 1**).

The researchers at Cell Genesys (which later spun off Abgenix, which was acquired by Amgen in 2007) and GenPharm (acquired by Medarex in 1997) both engineered mice by disabling the ability of mice to produce their own murine antibodies and replacing that function with human antibody genes.[16,17] These transgenic humanized mice were immunized with an antigen and, as a result, fully human antibodies were generated in these mice. The first fully human MoAb to be developed and marketed from one of these humanized systems was Vectibix, a human IgG2 antibody discovered using Abgenix XenoMouse technology in 2006, 12 years after the announcement of these engineered mice. These fully human MoAbs derived from humanized mice make up 25% of phase III clinical trials. Smith reported that peptides were able to be displayed as fusions of P3 on the tail fibers of *Escherichia coli* filamentous phage M13.[18] This method may be available broadly for the display of proteins including MoAbs; additionally, a phagemid system which allows for monovalent display is useful in selections of clones, especially for high-affinity binders rather than high-avidity binders.[19] This M13 P3-based phage display technology has become an optimal methodology for selection of antibody fragments capable of increased binding capabilities because antibodies function via binding ligands.[20] McCafferty et al. reported the bypassing of immunization by building a library of antibody genes, displayed on the P3 protein of M13 phage, using PCR techniques to recover the human gene from either B cells or hybridomas.[21] This method was followed by the construction of huge human libraries from either synthetic repertoires or from multiple naïve human donors.[22,23] The latter library from Cambridge Antibody Technology (CAT, a part of MedImmune, an owned subsidiary of Astra-Zeneca) was constructed using single-chain Fv constructs fused with a His and myc tag, produced under nM binders, and has become the prototype for many later libraries. A wide variety of strategies for making huge libraries or more focused libraries have been reviewed.[24] The first antibody from a phage-displayed human antibody library to be approved for therapeutic use was the anti-TNF alpha antibody Humira in 2002. Interestingly, Humira was not identified de novo from a human antibody library, but instead was identified via a guided selection method using murine antibody as the primary binder.[25]

Glycosylation of Therapeutic MoAbs

Glycosylation has become a focus of interest for medical biological investigators as a means to control the efficacy and safety of biological pharmaceuticals. MoAbs have a conserved glycosylation site at Asn297 on each CH2 domain of Fc, and in IgG from human serum these sites contain a family of diantennary complex-type oligosaccharides. The Fc glycans are highly heterologous due to variable processing of outer-arm monosaccharide residues onto the core heptasaccharide. Glycosylation at Asn297 is essential for stabilization of the CH2 domains and to achieve optimal antibody effector functions, including Fcg receptor and complement activation (**Table 1**). IgG glycoforms play a role in modulation of antibody effector functions. Recently, IgG-Fc bearing non-fucosylated oligosaccharides have been shown to enhance antibody-dependent cellular cytotoxicity (ADCC),[26] while IgG-Fc bearing sialylated oligosaccharides may modu-

late antibody-induced inflammation.[27] These properties may be exploited when developing antibody therapeutics to target tumors or to modulate inflammation in autoimmune diseases. Glyco-engineering of IgG provides possibilities for the production of therapeutic MoAbs with tailored effector functions.

Antibody Databases

To arrange and use the large amounts of data on immunology and its genetics, databases have been developed by IMGT (international ImMunoGeneTics)-ONTOLOGY.[28] The IMGT databases are standardized and combine genomic, genetic, and structural approaches, providing a bioinformatics of immunology essential for immunotherapy by allowing data comparison on antibodies. IMGT is available on the web at http://imgt.org. These databases are queried extensively and are used by scientists from both academic and pharmaceutical laboratories all over the world. The functions of the databases are 1) repertoire analysis of IgG antibody recognition sites in normal and pathological states such as autoimmune disease, infectious diseases, immunodeficiency, or hematological malignancies; 2) Ig repertoires in domestic and wild species; 3) genomic evolution research of adaptive immune responses; 4) structural evolution of Igs which interact with antibodies such as Fc receptors; 5) antibody engineering; 6) pathological diagnosis; and 7) therapeutic approaches.

Pharmacokinetics and Pharmacodynamics

The time course of therapeutic antibodies in the human body is determined by all processes involved in the absorption, distribution, metabolism, and excretion of antibodies. Despite the increasing number of therapeutic MoAbs in clinical use, certain aspects of the pharmacokinetic and pharmacodynamic properties of these MoAbs are not fully understood. Target-mediated disposition, in part, explains the characteristic non-linear pharmacokinetic and pharmacodynamic characteristics often observed for MoAbs. The interindividual variability of MoAb pharmacokinetics may be explained by interindividual variability with respect to target molecule expression and generation of immunological responses against administered MoAbs; among other sources, genetics and clinical status may explain interindividual pharmacodynamic variability. Pharmacokinetics and pharmacodynamics and related variability are explored through the use of pharmacokinetics and pharmacodynamics modeling approaches, which may be more complex than those of conventional small compounds. The half-life of IgG1, 2, and 4 in humans is approximately 21 days and that of IgG3, which has a different affinity to neonatal Fc-receptor, is 7 days. The half-lives of therapeutic MoAbs generally increase with the degree of humanization: murine (1.5 days) < chimeric (10 days) < humanized (12–20 days) = or < fully human (15–20 days).[29] The shorter half-life of murine MoAbs has been attributed to the lack of binding of murine IgG to human neonatal Fc-receptor and the generation of human anti-mouse antibodies (HAMA). The elimination of MoAbs is a complex multifactorial process involving protein catabolism, interaction with neonatal Fc-receptor, target-mediated elimination, immunogenicity, proteolytic degradation, and glycosylation.

Therapeutic MoAbs in Clinical Use

Since the first therapeutic MoAb OrthoClone OKT3 was approved in 1986 for the treatment of transplant rejection, many MoAbs have been developed and have become indispensable for some diseases. At least twenty-five different MoAbs were in clinical use by May 2009, and the current development pipeline contains more than 250 potential therapeutic MoAbs (**Table 1**).

1) MoAbs in oncology

CD20

CD20 antigen is expressed on normal B cells and on some neoplastic B cells. Rituximab, a mouse-human chimeric MoAb against CD20, was derived from mouse anti-CD20 MoAb IDEC-2B8 by engineering of human IgG1 and kappa constant regions and the original murine variable regions.[30] CD20 may function in normal B cell growth and activation and is expressed in more than 90% of non-Hodgkin lymphomas and 10% of chronic lymphocytic leukemias. Anti-CD20 MoAb may be an efficient treatment for B cell malignancies because CD20 is expressed at high levels on B cells, is relatively resistant to internalization, and is not shed, thereby allowing the MoAb to persist on the cell surface. Rituximab binds to an epitope on the large extracellular loop of CD20 with an affinity of 8 nM. The binding between CD20 and rituximab induces apoptosis and cell lysis via complement-dependent cytotoxicity (CDC) and ADCC.[30] Furthermore rituximab may sensitize the cells to the effect of chemotherapeutic reagents.[31] In most B cell lymphoma patients, rituximab causes a swift and profound depletion of circulating B cells which lasts for 3–6 months.[32] In a central trial investigating the safety and efficacy of rituximab for the treatment of relapsed or refractory non-Hodgkin B cell lymphoma, patients receiving four weekly doses of 375 mg/m^2 of rituximab intravenously had an overall response rate of 48% and a complete response rate of 6%.[33] Clinical studies of rituximab in relapsed or refractory CD20-positive non-Hodgkin B cell lymphomas showed that combination chemo/immunotherapy is superior to either treatment alone.[34] Rituximab was the first MoAb to be approved by the United State Food and Drug Administration (FDA) for treatment of malignancy.

Human epidermal growth factor receptor 2 (HER2)

Anti-HER2 MoAb trastuzumab, created by Genentech, was derived from murine MoAb mumAb4D5 by humanization of human IgG1 and kappa constant regions as well as human framework regions while retaining the complementarity-determining regions of murine mumAb4D5.[35] HER2 is a 185-kDa transmembrane receptor that is a member of the epidermal growth factor receptor family of receptor tyrosine kinases. While a ligand has not yet been identified for HER2, the receptor has been demonstrated to heterodimerize with other family members where it mediates signaling to the others. HER2 normally regulates cell growth and cell cycling via cyclin D and c-myc.[36] HER2 gene amplification and/or overexpression of HER2 occurs in approximately 20–30% of primary breast carcinomas and is associated with reduced overall survival.[37] Trastuzumab binds to an epitope in the juxtamembrane region of HER2 on breast carcinoma cells with an approximate affinity of 5 nM.[38] Trastuzumab functions as an anti-cancer reagent via direct inhibition of cell growth (receptor internalization, degradation, and decreased cell signaling) and indirect ADCC and CDC pathways.[39] Furthermore trastuzumab inhibits cleavage of an extracellular domain induced by metalloproteinase and induces apoptosis and impairs tumor angiogenesis.[39–41] In clinical study, patients who were administered with an intravenous loading dose of 4 mg/kg followed by 2 mg/kg weekly maintenance doses had an objective response rate of 15% and a median duration of response of 9.1 months.[42] In later phase II and III trials in metastatic breast carcinoma patients, trastuzumab combined with chemotherapy produced a better response rate, increased time to disease progression, and overall survival as compared with either chemotherapy or trastuzumab alone.[43] Interestingly, better response rates were seen in patients with overexpression of HER2 as compared to patients with normal expression.[44] A pharmacokinetic study of 4 mg/kg loading followed by 2 mg/kg weekly dosing showed that the mean half-life of trastuzumab was approximately 6 days.[42] However, a longer half-life of 16–18 days was seen in patients treated with 8 mg/kg loading followed by 6 mg/kg every 3 weeks.[45] Trastuzumab was approved in 1998 for treatment of breast cancer with HER2 overexpression. The indication of FDA approval recently was as adjuvant treatment of patients with HER2-overexpressing tumors with lymph node metastasis as part of a regimen of doxorubicin, cyclophosphamide, and paclitaxel. Trastuzumab is approved for use as a single reagent in patients with metastatic breast carcinoma whose tumors overexpress HER2 and who have received one or more chemotherapy regimens. Most adverse events in patients receiving trastuzumab are infusion-related, usually fever and chills. Other common adverse reactions are nausea, vomiting, diarrhea, rash, neutropenia, anemia, and myalgia. Serious toxicities of trastuzumab are cardiomyopathy, pulmonary damage, severe infusion reactions, and febrile neutropenia. Due to the risk of cardiomyopathy, it is recommended that patients undergo a thorough cardiac assessment before therapy and that cardiac function should be monitored throughout therapy.

CD33

CD33 is a type I transmembrane sialoglycoprotein that is a member of the immunoglobulin superfamily subset of immunoglobulin-like lectins.[46] CD33 is expressed on the cell surface of myeloid and sialic acid -binding early multilineage hematopoietic progenitor cells, monocytes, and blasts of acute myeloid leukemia (AML) (90%); however, CD33 is not expressed on normal pluripotent stem cells or non-hematopoietic cells.[47] Gemtuzumab ozogamicin (Mylotarg) is a humanized MoAb against CD33 conjugated with cytotoxic anti-tumor antibiotic calicheamicin. Gemtuzumab is derived from murine IgG1 MoAb P67.6 generated with human IgG4 and kappa constant regions as well as framework regions, while retaining complementarity-determining regions of the murine MoAb.[48] Gemtuzumab ozogamicin binds to the cell surface of CD33-positive leukemic cells with an approximate affinity of 0.08 nM. This MoAb functions as a vehicle delivering a conjugated toxic payload to CD33-positive leukemic cells. Binding of the MoAb to CD33 results in endocytosis, cleavage of the link between MoAb and calicheamicin, and release of calicheamicin.[49] The released calicheamicin is reduced by glutathione to form a reactive intermediate that binds to DNA, resulting in double-strand breaks and inducing apoptosis.[50] In a phase II study, 277 patients with CD33-positive AML received monotherapy with gemtuzumab ozogamicin in two doses separated by 2 weeks. As a result, 26% achieved remission, i.e., 13% of the patients had complete remission with platelet recovery and 13% had complete remission without platelet recovery.[51] The current FDA approved indications include treatment of patients with CD33-positive AML in first relapse who are more than 60 years old and who are not considered candidates for chemotherapy.

Vascular endothelial growth factor (VEGF)

VEGF is an angiogenic growth factor whose activity is mediated mainly by activation of VEGF receptor 2 [VEGFR-2 (Flk-1/KDR)], a high-affinity receptor tyrosine kinase. VEGF can also ligate other cell surface receptors including VEGFR1 and neuropilin-1 and -2. These receptors are located on the cell surface of the endothelium of blood vessels and lymphatic vessels. Stimulation of these cells by VEGF leads to cellular growth, inhibition of cell death, and new blood vessel formation.[52] VEGFR-2 is the main receptor through which the mitogenic and angiogenic effects of VEGF occur. There are several isoforms of VEGF: 121-, 165-, 189-, and 206-amino-acid isoforms can form by alternate splicing. VEGF functions in the regulation of both normal and abnormal angiogenesis,

including neoplasm and wound healing.[52] The production of VEGF is elevated in malignant neoplasm. It is secreted by tumor cells as well as by tumor-associated stromal cells.[53] The impairment of VEGF signaling by MoAbs, small molecules, or antisense oligonucleotides induces growth inhibition of tumor.[54,55] Bevacizumab, a humanized MoAb against VEGF, was derived from murine MoAb A.4.6.a against human VEGF165 by Genentech. Bevacizumab has human IgG1 and kappa constant regions as well as human framework regions, while retaining murine complementarity-determining regions.[56] Bevacizumab reacts and neutralizes all isoforms of VEGF effectively.[57] Bevacizumab binds an epitope of VEGF that is distinct from the receptor-binding site with approximate affinity of 2.2 nM.[58] In a xenograft model with human rhabdomyosarcoma and breast carcinoma, bevacizumab could inhibit tumor growth to under 10% of the original tumor weight and impaired tumor vasculature, decreasing microvessel density and permeability.[56,59] The FDA approved bevacizumab for the first-line treatment of metastatic colorectal carcinoma in 2004. Recent FDA approved indications include use in combination with 5-fluorouracil-based chemotherapy for first- or second-line treatment of patients with metastatic carcinoma of the colon or rectum. It is also indicated for use in combination with carboplatin and paclitaxel for the first-line treatment of advanced, recurrent, or metastatic non-squamous cell, non-small-cell lung carcinoma. Recently, r84, a fully human MoAb against VEGF, was reported. r84 binds human and mouse VEGF and selectively blocks VEGF from interacting with VEGFR2, but it does not interfere with VEGF-VEGFR1 interaction.[60]

2) MoAbs in autoimmune and inflammatory disorders and infectious diseases

Tumor necrosis factor (TNF) alpha

TNF alpha is a proinflammatory cytokine known as a mediator of chronic inflammation and cachexia. The anti-TNF-alpha MoAb infliximab (Remicade) was developed as a human/murine chimeric IgG by Centocor and may be used for the treatment of autoimmune diseases such as rheumatoid arthritis, Crohn's disease, ulcerative colitis, psoriasis, and ankylosing spondylitis.[61]

Infliximab ligates to TNF alpha with an affinity of 1.8 nM and strong avidity; it thus effectively prevents TNF alpha from binding to its receptors.[62] Infliximab neutralizes all forms of TNF alpha, including soluble, transmembrane, and receptor-bound forms; in contrast, the TNF-alpha antagonist Enbrel, in the format of a recombinant FC fusion protein, neutralizes only soluble TNF-alpha and beta . Infliximab is administered by intravenous infusion and has a half-life of 8–10 days.[63] The clinical effect of infliximab was obvious from the rapid reduction of proinflammatory cytokines and other inflammatory markers. Infliximab has a safety profile, and infusions are generally well tolerated. Serious adverse events include an increased risk of tuberculosis, opportunistic infections, or worsening of preexisting heart failure. The other MoAb against TNF alpha, adalimumab (Humira), was developed as a fully human MoAb by Cambridge Antibody Technology.[64] Adalimumab was derived from murine antibody MAK195 using the guided selection phage display method of Cambridge Antibody Technology. This method involves a series of selections of the human heavy chain and light chain variable region by pairing them with the variable regions of MAK195; these variable domains were fused with the human IgG kappa constant domain. The fully human MoAb clone D2E7, produced in CHO cells, has a binding affinity of 100 pM to TNF alpha.[65] Adalimumab is administered subcutaneously at 40 mg every 1 or 2 weeks for rheumatoid arthritis patients. The maximum serum concentration and the time to reach the maximum concentration were 4.7 μg/mL and 131 h, respectively, following a single 40-mg subcutaneous injection to healthy adult human volunteers. The average absolute bioavailability of adalimumab, evaluated following a single 40-mg subcutaneous dose, was 64%. The mean serum half-life was 10–20 days for intravenous infusions.[66] In patients with rheumatoid arthritis, a dramatically decreased level of inflammatory markers such as C-reactive protein, fibrinogen, and cytokines was observed. Adalimumab is a fully human MoAb; however, an immune response against adalimumab is still present in 5% of infused patients. It is marketed both as preloaded syringes and as preloaded pen devices for self-injection by patients. Recently, a new fully human MoAb against TNF alpha, golimumab, was developed by Centocor. Golimumab is a human IgG1 (VH3 and V kappa 3) generated using Medarex's UltiMAb technology. Its variable domains are almost 100% identical to the human germline sequences and its constant domain is identical to corresponding regions of infliximab in terms of amino acid sequences.[67,68] It was approved by the FDA in April 2009, alone or in combination with methotrexate, for the treatment of moderate to severe rheumatoid arthritis, active psoriatic arthritis, and active ankylosing spondylitis. Unlike infliximab, which is administered through intravenous infusion, golimumab has been approved for subcutaneous injection once a month, which is a less frequent dose than two other leading biologic reagents, etanercept and adalimumab, in the class of anti-TNF alpha therapy.

Respiratory syncytial virus (RSV)

RSV is classified in the genus *Pneumovirus*, belongs to the Paramyxoviridae family, and caused of serious respiratory tract diseases in children and infants. Over 70% of infants are infected in the first year after birth, and virtually every infant has experienced RSV infection by their second birthday.[69] RSV infection causes

about 4500 deaths and the hospitalization of about 90,000 children every year in the United States. The humanized MoAb against the F protein of RSV, palivizumab (Synagis), was derived from a selected murine MoAb using a CDR-grafting-based humanization method.[70] The original murine MoAb, mAb 1129, was selected because it recognized the antigenic site A on the F protein of RSV.[71] Palivizumab has a binding affinity of 3.46 nM to the F protein of RSV using Biacore analysis, and its half-life is 17 days in humans.[72] Palivizumab has been shown to cut the chance of RSV high-risk infant hospitalization by 50%. Palivizumab was the first MoAb reagent to treat an infectious disease.

3) A candidate novel MoAb therapy in oncology and autoimmune and inflammatory disorders

CD26 is a 110-kDa surface glycoprotein with dipeptidyl peptidase IV activity able to cleave selected biological factors to alter their functions.[73] CD26/dipeptidyl peptidase IV is involved in T-lymphocyte costimulation and signal transduction processes.[74] CD26 is involved in the development of certain human cancers.[75] It was found that CD26 is a collagen-binding protein using a CD26-positive JMN cell line, which is derived from malignant mesothelioma.[76] Several studies have shown that anti-human CD26 murine MoAb inhibits growth of CD26-positive T-cell malignancies and renal cell carcinoma.[77,78] YS110, a humanized MoAb against CD26, was developed from murine MoAb 14D10 against human CD26 by Y's Therapeutics. YS110 has human IgG1 and kappa constant regions as well as human framework regions, while retaining partially murine complementarity-determining regions using an in silico humanization method. YS110 induces cell lysis of malignant mesothelioma cells via antibody-dependent cell-mediated cytotoxicity in addition to its direct anti-tumor effect via $p27^{kip1}$ accumulation. In vivo experiments with mouse xenograft models involving human malignant mesothelioma cells showed that YS110 treatment drastically inhibits tumor growth in tumor-bearing mice, resulting in enhanced survival.[79] YS110 may have potential clinical use as a novel cancer therapeutic agent in CD26-positive cancer including malignant mesothelioma, lymphoma, and renal cell carcinoma. Furthermore, it was shown that YS110 induced a translocation of CD26 from cell membrane to nucleus within 1 h, and intra-nuclear CD26 may function as a growth inhibitor.[80] On the other hand, CD26 expression on T lymphocytes is enhanced after activation of T cells, while it is preferentially expressed on a subset of CD4+ memory T cells in the resting state. The binding of anti-CD26 MoAb inhibits human T-cell growth and proliferation in both CD26-transfected Jurkat T-cell lines and human T-cell clones by inducing G1/S arrest, which is associated with enhancement of $p21^{Cip1}$ expression.[81] YS110 may have potential use in the clinical setting involving activated T cell dysregulation, including autoimmune disorders and graft-versus-host disease.[81] YS110 was approved as an Investigational New Drug by the FDA and is currently at the phase I clinical trial stage.

4) Adverse effect of MoAbs in a clinical trial of immunotherapy

The first phase I clinical trial of TGN1412, a novel superagonist anti-CD28 monoclonal antibody that directly stimulates T cells, was carried out in 2006. Within 90 min after receiving a single intravenous dose of the drug, all six volunteers had a systemic inflammatory response characterized by a rapid induction of proinflammatory cytokines and accompanied by headache, myalgia, nausea, diarrhea, erythema, vasodilatation, and hypotension. Within 12–16 h after infusion, they became critically ill, with pulmonary infiltrates and lung injury, renal failure, and disseminated intravascular coagulation. Severe and unexpected depletion of lymphocytes and monocytes occurred within 24 h after infusion. All six patients were transferred to an intensive care unit where they received intensive cardiopulmonary support, dialysis, steroids, and an anti-interleukin-2 receptor antagonist antibody. Despite evidence of multiple cytokine-release syndrome, all six patients survived.[82] Stebbings et al. reported novel in vitro procedures in which TGN1412, immobilized in various ways, is presented to human white blood cells in a manner that stimulates the striking release of cytokines and profound lymphocyte proliferation that occurred in vivo in humans. These procedures would have predicted the toxicity of this superagonist and are being applied to emerging immunotherapeutics.[83]

Summary

Thirty therapeutic MoAbs have been approved around the world, including 24 in the United States, several of which have attained blockbuster status with sales reaching the coveted billion-dollar mark and beyond. Five MoAbs, rituximab (Rituxan), infliximab (Remicade), bevacizumab (Avastin), trastuzumab (Herceptin), and adalimumab (Humira), generated sales of over $4 billion each in 2008, and global sales for this entire sector surpassed $30 billion in that year. There are more than 250 therapeutic MoAbs now undergoing clinical trials, mostly concentrated in the areas of malignancy, autoimmune and inflammatory diseases, and infectious diseases. Beyond these, hundreds more candidates are already at the preclinical stage of development. Dimitrov et al. suggested that there have been two major eras in antibody discovery: the original serum therapy period of the early 1900s and today, in which significant changes are impacting the way investigators design and make thera-

peutic proteins.[84] In the next era, therapeutic MoAbs may be broadly developed using novel concepts or methodologies such as 1) extensive searches of target molecules, 2) new protein engineering, 3) generation of improved drug delivery systems, and 4) research on direct functions of MoAbs against cells other than by ADCC or CDC.

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