chapter two

*Site-specific drug delivery utilizing monoclonal antibodies**

Contents

I. Introduction

At the beginning of this century, Paul Ehrlich reported the discovery of antibodies.[1] Since that time, many investigators have done extensive work using a wide variety of antibody molecules in immunocytochemistry, radioimmunoassay, and clinical medicine. In 1976, Kohler and Milstein employed a method of somatic-cell hybridization in order to successfully generate a

* Adapted from Ranade, V.V., Drug delivery systems. 2. Site-specific drug delivery utilizing monoclonal antibodies, *J. Clin. Pharmacol.*, 29, 873, 1989. With permission of the *J. Clin. Pharmacol.*, and J.B. Lippincott Publishing Company, Philadelphia, PA.

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continuous "hybridoma" cell line capable of producing monoclonal antibody (MAb) of a defined specificity.[2] Subsequently, several MAbs have exhibited specificity for target sites. It is this property of MAbs that makes them excellent candidates as carriers of therapeutic agents for delivery to specific sites.[3,4]

A. Chemistry

Antibodies are complex proteins, consisting of multiple polypeptide chains that contain a variety of reactive chemical groups, such as amino, carboxyl, hydroxyl, and sulfhydryl. Functionally, MAbs possess a molecular polarity based on the joining of an antigen-binding fragment (Fab) to a complement-fixing fragment (Fc). The Fab fragment is responsible for specific antigen binding, whereas the Fc fragment binds to effector cells, fixes complements, and elicits other *in vivo* biological responses.

In order to obtain a MAb suitable for the treatment of human disease, it is necessary to maintain both the physical and functional properties of the antibody throughout the steps of production, isolation, purification, and modification. Antibody modification, performed to increase theoretical efficacy, can consist of conjugation of the protein to the following: radionuclides (e.g., ^{131}I and ^{111}In), chemotherapeutic drugs (e.g., methotrexate and vinblastine), and polypeptide toxins (e.g., ricin A chain and polkweed antiviral protein [PAP]).

B. Polyclonals vs. monoclonals

Antibodies can be heterogeneous with respect to size, charge, antigen specificity, and affinity. These factors may be significant when antibodies are used as a drug delivery system, either alone or when conjugated. For example, some antibody molecules may be degraded rapidly and excreted while others may have longer half-lives.[3–5] Earlier researchers used polyclonal antibodies for drug targeting.[6–8] However, polyclonals contain an inherent deficiency due to lack of specificity that is further compounded by the fact that reproducibility within polyclonal antisera was not always obtained. In view of these problems, researchers continued to focus on developing MAbs with their attendant increased specificity.[9]

Polyclonal antibodies may offer potential advantages in drug delivery, such as recognition of more than one specific location at a given target site. However, this can also be achieved by using mixtures of MAbs of desired specificity. A wide range of animal species can be used to produce polyclonal antibodies, which is a distinct advantage. At the present time, production of MAbs is predominantly limited to mice, rats, and, to some extent, humans.[10]

C. Conjugation of antibodies

Drug targeting and delivery using antibodies has been most useful in the field of chemotherapy[11,12] because this is an area of research in which there is the greatest need for target-site specificity. Anticancer drugs, in particular, often display high toxicity, and they frequently have a low therapeutic index.[2,13,14]

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Early work attempting to conjugate polyclonal antibodies with anticancer drugs involved simple covalent-bond coupling. For example, in 1958, Mathe et al.[15] described the conjugation of methotrexate to antimouse leukemic antibodies for drug targeting. Nearly 15 years later, chlorambucil was coupled to polyclonal goat or rabbit antitumor antibodies.[16] Drug-targeting studies followed using rabbit antibodies against a mouse lymphoma coupled to drugs such as chlorambucil, methotrexate, melphalan, daunomycin, and adriamycin. In similar studies, drug-polymeric carrier complexes have also been coupled to an antibody. Researchers working at the Weizmann Institute in Rehovot, Israel devised[17] such polymeric carriers and were successful in coupling methotrexate, cytosine arabinoside, and platinum to polyclonal, as well as MAbs, against both animal and human tumor targets.[18]

During the period between 1980 and 1988, hybridoma technology was developed. Through its use, complex molecules, such as histocompatibility antigens, developmental and differentiation antigens, tumor-specific antigens, serum proteins, hormones, neurotransmitters, and various kinds of receptors, were recognized, isolated, purified, quantified, and biochemically characterized, and their respective antibodies made available for targeting to specific sites.[11,19–21]

II. Production of monoclonal antibodies

Initial success achieved using hybridoma technology led to further expansion of immunoconjugate-based targeting, especially in detecting and treating human cancers.[22] Rowland described a technique for identifying antigens associated with hematological malignancies. He also emphasized that the choice of normal cells and the method of screening are important in the testing of antibodies for therapeutic immunotargeting.[23–26] The widely accepted method for the production of monoclonals for anticancer targeting is that described by Brown and co-workers[27] in which the appropriate type of malignant cell is used as the immunogen. Hybridomas utilizing hybridoma technology are routinely made in stepwise conventional small-scale culture procedures, as is briefly described in the following.[28,29]

1. The antigen, a foreign substance, such as a lung cancer cell, is injected into a mouse. The mouse's immune system recognizes the lung cancer cell as foreign and directs the spleen to produce specific antibodies to attack that antigen. The spleen is then removed, and the antibody-producing cells are collected.
2. Myeloma cells are isolated from a mouse tumor. These cells have the ability to reproduce continuously in the laboratory.
3. Spleen and tumor cells are fused together to form "hybridomas." A drug is added to kill the tumor cells that do not fuse. The surviving hybridomas have the spleen cell's ability to produce antibodies and the tumor cell's ability to reproduce.

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4. Each hybridoma is isolated and allowed to grow into a large colony of cells that produce a single MAb.
5. Each MAb is screened for its ability to attack the original cancer cells, and the hybridomas producing the desired antibody are kept.
6. The desired hybridoma cells are injected into a mouse where they form a tumor that produces large amounts of concentrated antibody.

The first critical step in generating a therapeutic or diagnostic MAb — after initial isolation — is to produce the antibody product. Damon Biotech of Needham Heights, Massachusetts, has used a microencapsulation process developed to produce significant quantities of therapeutic MAbs. Known as the Encapsel method, this gentle chemical process results in the formation of a semipermeable membrane around a group of hybridoma cells. Within the microcapsule membrane, these cells proliferate rapidly and secrete the MAb. The antibody is harvested from the intracapsular space at the end of a 2- to 3-week culture period.

A. Continuously proliferating cell lines

Numerous studies have demonstrated that continuously proliferating cell lines can produce human antibodies of predetermined specificity. These lines have been established as a result of infecting peripheral blood lymphocytes with the Epstein–Barr virus (EBV). This approach has proved to be of limited potential use, however, because all EBV-transformed cell lines decline in antibody production over time. Production of rodent MAbs against a wide variety of antigens has recently been reviewed.[28]

B. Human–human hybridomas

The availability of appropriate human myeloma lines can facilitate production of human hybridoma products, since in these human–human hybrids, repression of human chromosome function is minimal. Intensified research efforts have been made to obtain a drug-sensitive human myeloma cell line capable of fusing with human B-lymphocytes. Olsson and Kaplan have reported the establishment of human–human hybridomas that produce MAbs against the hapten dinitrophenol.[30]

C. Large-scale production

The need for a successful large-scale MAb production technique is indicated by the growing commercial market for antibody-based products and the increased importance of *in vivo* diagnostic as well as therapeutic applications.[31] According to market research, total sales of *in vivo* and *in vitro* MAb-based products reached approximately $8 billion in 1993, and this volume is expected to increase in subsequent years. Therefore, significant commercial production of MAbs is emphasized and Cortesis and Proby[32]

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have described the scale-up production of MAbs. In their procedure, MAb-producing murine hybridomas were cultured in 2-L and 40-L working-volume airlift bioreactors. Batch and semicontinuous culture protocols were used successfully, and ultimate cell density, viability, and monoclonal antibody productivity using these systems were found to compare favorably with results obtained by the conventional small-scale culture method described previously.[33,34]

III. Drug-monoclonal antibody conjugates for drug targeting

A. Principles

Use of MAbs in targeting cytotoxic drugs to specific tissues has been studied for over 20 years.[35–37] Antibodies have been found to have many applications in the management of human carcinomas, including colorectal, gastric, ovarian, endometrial, breast, lung, and pancreatic.[38–40] Schlom, in his articles on cancer therapy, has compiled a list of considerations when assessing the use of MAbs to treat cancer.[41,42] These are summarized as follows: number of antigen molecules per cell surface; number of cells expressing the reactive antigen in the tumor mass; size of the tumor mass; fate of the antigen-antibody complex (stability on cell surface, internalization, capping, shedding); degree of tumor vascularization; degree of tumor mass infiltration and necrosis; presence and reactivity of circulating antigen in the blood; duration of MAb binding to cell surface; isotype of immunoglobulin (IgG subtypes or IgM); species of immunoglobulin (murine, human, or chimeric recombinant); whole immunoglobulin or fragments (Fab, Fab', $F(ab')_2$); clearance of MAb from blood, excretion, or reticuloendothelial system; dose of MAb used; route of inoculation of MAb (intravenous, intraperitoneal, intralymphatic, or intraarterial); and development of a human immune response to the administered MAb.

If a radiolabeled MAb is used, consideration has to be given to factors such as the ability of the MAb to be labeled with specific radionuclide, specific activity of the radiolabeled MAb, affinity of radiolabeled MAb, depth of tumor from body surface (for tumor localization), time of scanning (for tumor localization), choice of radionuclide, method of linkage of radionuclide to MAb (metabolism and catabolism of MAb-radionuclide complex), and dose fractionation of administered MAb.[43]

Widder et al.[44] have enumerated several requirements for an ideal carrier. The essential requirements are illustrated schematically in Figure 2.1.

B. Drug antibody bonding

For drug targeting using antibodies, it is important that the drug and the antibody retain their respective activities and that the conjugate remains stable

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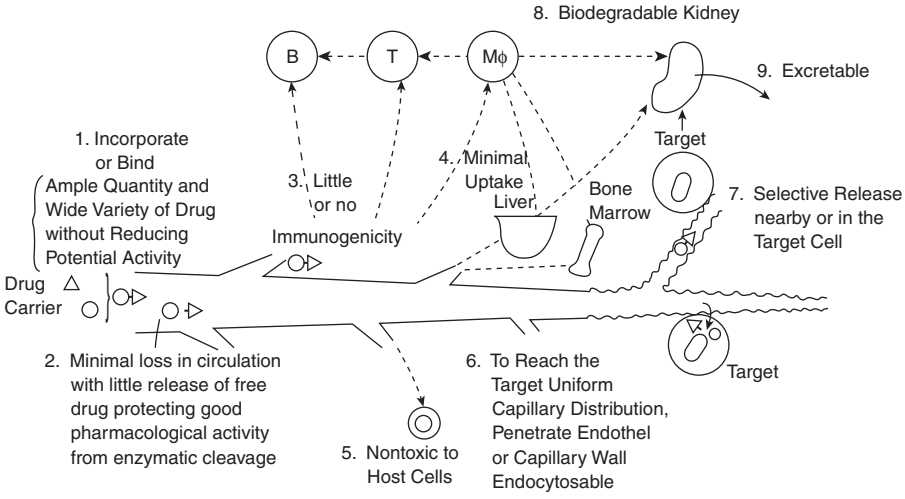

Figure 2.1 Schematic illustration of requirement for drug carriers. (From Bruck, S.D., Ed., *Controlled Drug Delivery*, Vol. II, *Clinical Applications*, CRC Press, Boca Raton, FL, 1983.)

in transit to its target site. In this regard, it is possible that chemical coupling methods may be too drastic or create bonds that are not stable *in vivo*.[26]

Lysine residues occur abundantly in immunoglobulins, with the epsilon amino side chain, the commonly preferred site for drug conjugation.[45] Binding of a drug to the epsilon amino group of the immunoglobulin near its carboxylic acid group forms a carboxamide bond. If the drug's carboxylic acid group is not responsible for its pharmacological action, then conjugation should not affect efficacy. In studies using chlorambucil, the formation of an ionic complex and not a covalent link is also possible. Gallego et al. have reported that a cis-aconityl linkage gives rise to a stable conjugate in the case of daunomycin and amino sugars.[46]

Drawing general conclusions about drug-to-antibody coupling methods when using monoclonals is fraught with difficulties since one monoclonal antibody may behave quite differently from another. This is evident from the studies using an active azide derivative of a vinca alkaloid to produce vindesine-monoclonal antibody conjugates.[47] It has also become clear that when highly homogeneous monoclonal preparations are used in experiments, each antibody needs to be evaluated individually for any particular type of drug-coupling procedure requiring chemical manipulations.[47] Similar conclusions have been reached when attempting to couple cytosine arabinoside to a MAb recognizing a human T cell.[48]

C. In vitro *and* in vivo *testing*

Research workers in the field of immunoconjugate targeting have predominantly used *in vitro* test systems to evaluate the potential therapeutic value

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of their preparations. The advantages of *in vitro* test systems are that many variables can be evaluated for drug targeting using small quantities of conjugate over wide ranges, results can be obtained in a short period of time, and many different target cells can be used. It is known that many drug-antibody conjugates, although highly specific, are less potent than the free drug when tested on cells *in vitro*. When tested *in vivo*, loss of potency can be compensated for by a longer target-site residence time. Studies using vinca alkaloids coupled to monoclonals recognizing human tumor-associated antigens clearly demonstrate this point.

The vincas were originally chosen for coupling because of their high molar potency. It appeared likely that if drug potency could be fully retained in immunoconjugates, then effective doses could be delivered even though the site density on the target cell was not high. An extensive study of vindesine conjugated to monoclonal anti-CEA (carcino-embryonic antigen) has been carried out in which nine different human cell lines were examined for target cytotoxicity *in vitro*. In these studies, it was found that free vindesine was considerably more potent than the conjugate. However, it was also found that conjugates did not affect cells lacking the target antigen CEA, whereas free vindesine failed to discriminate between them.[49,50]

Obviously, a major objective of drug delivery using antibodies is *in vivo* therapy. Therefore, it is important to test preparations *in vivo* even if the *in vitro* potency of conjugates appears poor in comparison with the free drug. In this regard, vindesine conjugated to anti-CEA MAb has been found effective in suppressing a human CEA expressing tumor xenograft in athymic mice.[51,52]

The *in vivo* selectivity of vindesine anti-CEA on cells expressing target antigen can be demonstrated in two ways. First, by reduced systemic toxicity giving rise to an improved therapeutic index, and second, by lack of effect on a tumor not expressing CEA. These efforts support the view that targeted site-specific drug delivery has been achieved.[53] However, direct evidence has not been available until recently. To overcome this aspect, a series of experiments was carried out in which the *in vivo* distribution of a radiolabeled drug was determined in tumor-bearing mice using either free vindesine or vindesine conjugated to monoclonal anti-CEA or to an irrelevant monoclonal. The results confirmed that drug delivery was target-site selective when the anti-CEA antibody was used. When tumor- or tissue-to-plasma ratios of a labeled drug were examined, it was found that up to ten times as much drug accumulated in the tumor as normal tissues when delivered in the form of a specific antibody conjugate. In contrast, no selective uptake was observed with either a free drug or a conjugate-involving irrelevant antibody.

Several aspects of these findings are of considerable importance when labeled drugs are used.[54,55] First, the concentration of drug accumulated at the target site remains high for several days. This is probably due to the long biological half-life of the antibody. Second, the high tumor selectivity of the conjugated drug is observed over a wide range of dose levels. From these experiments, it has been possible to calculate the amount of drug delivered to the tumor in its conjugated form as compared to free drug. It appears that

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increased quantity of drug delivered to the tumor as an antibody conjugate may compensate for apparent loss of potency due to conjugation. According to Rowland,[12] this may explain why the *in vivo*-effective dose levels of free and conjugated drug are more similar than those obtained *in vitro*.

As cited by Rowland in his studies,[12] although the dose of conjugated vindesine that can be administered is considerably higher than that of free drug in studies using human tumor xenografts, it is important to determine the acute toxicity from which an LD_{50} dose can be calculated. In one study, the type of toxicity normally associated with many anticancer drugs, namely bone marrow depression, damage to cells of the gastrointestinal mucosa, or neurotoxicity, was completely absent from mice treated with high doses of conjugated vindesine. This suggests, therefore, that toxicity reduction of many drug-antibody conjugates may be the result of uptake by the reticuloendothelial system (RES).[12] Studies utilizing radiolabeled vindesine-antibody conjugates do indicate an accumulation of drug in the liver and spleen.

Polyclonals and MAbs have been used in radioimmunoimaging in recent years. Ford et al.[56] used a conjugated vindesine and polyclonal sheep anti-CEA preparation that had been shown previously to localize in tumors of patients with gastrointestinal malignancies. This study demonstrated that radiolocalization of the antibody was still possible despite the presence of the conjugated drug. Therefore, it may be possible that an antibody capable of imaging a patient's tumor will also deliver a drug, such as vindesine, to the target site. In one patient with high circulating levels of CEA, tumor biopsy after injection of iodine-labeled antibody conjugate showed nearly five times the radioactivity in the tumor than in surrounding normal tissue. Thus, the presence of circulating CEA did not prevent localization of conjugate.[57]

Antibody toxin conjugates *in vitro* have been found to be highly selective in killing cells bearing the appropriate antigen. For example, Blythman and co-workers have found that murine MAbs of the IgM class directed against the Thy 1.2 differentiation antigen of mouse T cells, when coupled with ricin-A chain, killed mouse leukemia cells carrying the same antigen.[58,59] Cytotoxic activity of the MAb and toxin-subunit conjugate, called immunotoxin, was specific since it did not have an effect against Thy 1.1 cells. Corresponding studies carried out *in vivo* demonstrated prolonged survival time in mice treated with immunotoxin and suggest the potential use of immunotoxin as a highly sensitive test system for studies relating to the treatment of cancer. In the past, several investigators have attempted to use antibodies to target the toxic activity of cytotoxic drugs to tumor cells, but lack of high-titer antibody against specific cell-surface antigens has been a major limiting factor in their use. With these antibodies, it is possible to know which class, subclass, or antibody fragments may be more efficient in promoting the productive internalization of covalently coupled toxin.[60]

Hybridoma antibodies have been used successfully to enhance renal allografts in rats.[61,62] This is the first *in vivo* demonstration whereby passively transferred allo-antibodies against major histocompatibility determinants were fully capable of inducing a state of immunological enhancement.

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Cosimi et al.[63] have shown that a MAb to OKT4, an antigen on T inducer cells, had an immunosuppressive effect when given to monkeys and produced prolonged kidney graft survival. Results obtained with conventional antisera have suggested a potential role for antibody therapy, but the success of this approach has been difficult to assess because of the limited quantities of high-titer antibody of appropriate class, affinity, and specificity.[64,65]

Bernstein et al. have used a MAb against a normal differentiation antigen (Thy-1) for the treatment of murine leukemia of spontaneous origin.[66] A MAb-to-rabies virus has been shown to be protective in mice against infection with lethal doses of rabies virus.[66]

Possible application of MAbs in direct therapy against parasites is also under investigation.[67,68] In this respect, two potential kinds of roles can be envisioned for MAbs: first, monoclonal antibody-cytotoxic drug conjugates could be used to carry the drug to the target parasite, thus concentrating the drug's effect. Second, it may be feasible to produce MAbs against parasite antigens that will themselves find and attack the invading parasites, thus intervening in the life cycle of the parasitic organism.

The potential of MAbs in combating malaria has been shown by two research groups.[67,69] One group developed MAbs to surface antigens of *Plasmodium berghei*, which interfered with rodent malaria infection at the sporozoite or marozoite stage. One MAb was of the IgG subclass directed against a protein antigen (Pb44) that is present on the surface membranes of sporozoites. This antibody abolished the infectivity of the parasites in both *in vivo* and *in vitro* studies. Perrin et al. have reported that a monoclonal antibody against *Plasmodium falciparum* can inhibit infection by this parasite in cell cultures of human erythrocytes.[69]

An extensive study of a spectrum of MAbs against *P. falciparum* will help to define antigens required for protection and effective mechanisms of immunity. With the use of these MAbs, it will be possible to purify potentially protective antigens of *P. berghei*, *P. falciparum*, and other parasites. However, because obtaining large quantities of *Plasmodium* antigens is not practical, it may be desirable to utilize recombinant technology to produce defined malarial antigens. In this respect, MAbs will be extremely useful for identifying bacterial colonies secreting the desired antigens. MAbs specific for schistosomal antigens, *Theileria parva*, and *Toxoplasma gondii*, which can be used diagnostically, have also been reported.[69–72]

Table 2.1 presents a partial list of MAb-technology-based research and development firms, their products, and usage.[73]

IV. Recent studies with monoclonal antibodies

A. Highlights of current research

Several investigators have prepared "second-generation" MAbs. In this process, the MAb first evaluated was used to purify the target antigen, which was then used as an immunogen to prepare a new generation of MAbs that

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Table 2.1 Monoclonal antibodies

Corporation	Product	Usage
Damon Biotech	Encapsel Technology encapsulating living insulin producing cells	Production of insulin
Monoclonal Antibodies, Inc.	Ophthalmological diagnostic tests	Identification of agents responsible for ocular infections
Ortho Pharmaceutical Co.	Orthoclone OKT3	Prevent rejection of new kidney
Centocor	HA-1A Centoxin endotoxin MAb Myoscint	Gram-negative sepsis Septic shock Detect spread of melanoma, thrombolytic therapy, cardiac imaging
Biogen	CD4 agent	In AIDS
NeoRx	Oncotrac	Detect spread of melanomas
Genentech	CD4 agent Oncogene	In AIDS Breast and ovarian cancer
Cetus	Prolenkin (interleukin-2)	Anticancer
Amgen	Epogen (Erythropoeitin)	Stimulates RBC formation
IDEC	3C9 Murine anti-idiotype inhibitor	In HIV-infected patients
Cytogen	MAbs targeting, breast, colorectal, and ovarian tumors	Anticancer Imaging agents
Xoma	E5 (Xomen) Monoclonal IgM anti-antibody against endotoxin XomaZyme H-65 and 791	Septic shock Gram-negative sepsis for transplant situations and autoimmune diseases
Allergen	Regulatory anti-ALG-991 (Murine MAb)	To modulate allergic disorders (e.g., asthma, allergic rhinitis, poison ivy, and oak allergies) To reverse sensitivity to urushiol in mice
Immunomedics	ImmuRAID-AFP	Detect germ cell tumors
Cell Genesys	Developing technology to produce human–human MAbs in mice	For transferring human genes into mice and to map the human genome
Cantab	LM.CD45	Used in kidney transplants

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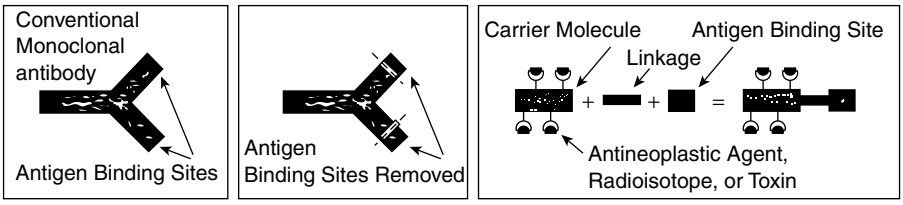

***Figure* 2.2** Second-generation monoclonal antibodies. (Reprinted with permission from *Drug Topics*, Medical Economics Co., June 2, 1986.)

were reactive with that molecule. However, *a priori* reasons exist for the assumption that the first MAb directed against a given tumor antigen will be the best. Amino acid sequence data obtained from purified antigen of DNA sequences obtained from cloned genes that code for these antigens provide sufficient information for the preparation of synthetic peptides and the subsequent development of MAbs of predefined specificity (see Figure 2.2).

A conjugate of a MAb and the anticancer agent desacetyl vinblastin has been found to recognize lung, colorectal, breast, ovarian, and prostate tumors.[74] MAbs have also been used in trials designed to control the common cold. In this case, the MAbs do not attack the cold virus directly. Instead, they interact with receptors on the surface of the epithelial cells lining the nasal passages. By blocking these receptors, the MAbs prevent viral entry.[75]

Antigenic heterogeneity has been a major consideration in the therapy of solid tumors. Unlike many antigens that are associated with leukemias, lymphomas, and melanomas, many of the oncofetal antigens associated with pancarcinomas are not always expressed in all cells within a given tumor mass. Studies have demonstrated that recombinant α-(clone A), β-ser, and γ-interferons can regulate the expression of certain tumor-associated antigens, such as CEA and TAG-72. These studies have also shown that when cells do not express CEA or TAG-72 — as in the case of normal cells and noncarcinomas, such as melanoma — the exposure of these cells to recombinant interferons does not affect antigen expression.

It has also been reported that interferons can up-regulate tumor targeting of radiolabeled MAbs in an *in vivo* animal model and in clinical trials. Preclinical studies have demonstrated that recombinant interferons can increase both the amount of tumor antigen expressed by a given tumor cell and the percentage of tumor cells that express the antigen. Thus, together with MAb combinations, radionuclides can kill several cell diameters, and the use of recombinant interferons and antigenic heterogeneity of tumor masses can be addressed.[74]

I^{125}-labeled MAb B72.3 has been used in radioimmunoguided surgery (RIGS) to localize up to 70% of colorectal carcinoma lesions. Significantly, RIGS also reportedly identified tumors not detected by conventional surgical procedures in 20% of the cases. The RIGS diagnostic procedure also identifies those patients whose tumors are targeted by a given MAb; therefore, the

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procedure can be used to select patients who are more likely to respond to a specific MAb therapy.[76]

Another drug that has been experimentally piggybacked on MAbs is urokinase, the thrombolytic agent. Urokinase is not a clot-specific agent — it causes the breakdown of fibrinogen, a property that leaves open the possibility of major bleeding problems in patients. Laboratory workers have now succeeded in attaching urokinase to antibodies against fibrin.[77]

The method for dissolving blood clots that cause myocardial infarction is based upon a specially designed MAb that activates clot-dissolving chemicals only at the site of the clot. In theory, the new technique should dissolve blood clots with less risk of bleeding occurring in other parts of the body, as can happen with current clot dissolvers. It also could reduce or even eliminate the use of manufactured clot dissolvers, utilizing instead clot-dissolving substances naturally present in the body.

The basic strategy behind this is to take the antibody that interacts with fibrin and use it to concentrate the natural clot dissolver directly on the clot. One major natural clot dissolver is known as tissue plasminogen activator, or TPA. TPA activates plasminogen that ordinarily lies latent in the blood. Once activated, plasminogen triggers a chemical chain reaction that destroys fibrin and dissolves the blood clot. Current artificially produced versions of the clot dissolvers are infused into the bloodstream where they promote a freer flow of blood throughout the body. However, this is done at the risk of causing hemorrhage, something that should be minimized by the antibody, which should trigger the clot-dissolving reaction only in the vicinity of the clot.[78]

Other examples of studies using MAbs include agents such as immunoabsorbents, hypolipemics, cytokines, porphyrins, antiferritin, and Techniclone (Lym-1). In addition, significant products involving the use of recombinant technology and genetic engineering are: Recombivax HB (a recombinant hepatitis-B vaccine), kidney plasminogen activator, Eminase (anisylated-plasminogen-streptokinase-activator complex), alpha-2 interferon (Intron A), alpha-A interferon (Refron-A), beta interferon (Betaseron), alpha-1 antitrypsin (AAT), and Activase (recombinant version of t-PA).[78]

The IgG murine MAb Alz-50 has been derived from a mouse immunized with homogenates of postmortem ventral forebrain tissue from four patients with Alzheimer's disease. Hybridoma cell-culture supernatants were initially screened based on the comparison of their binding to Alzheimer's brain homogenates immobilized onto polyvinyl plates with identically prepared control homogenates. Alz-50 was described as recognizing an antigen in the affected region of the Alzheimer brain that was elevated 15 to 30 times. Immunocytochemical analysis of the antibody revealed it labeled Alzheimer neurofibrillary tangles, as well as selective neuronal populations.[79]

Many procedures have been reported for coupling anthracycline drugs to an antibody for drug targeting. A recent report describes a new coupling procedure that uses an activated daunorubicin derivative that is later added to the antibody. Utilizing this procedure produced no significant polymer-

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ization of the conjugate and a full recovery of pharmacological activity as tested *in vitro* on CEA-producing human colon adenocarcinoma cells. Activated drug was found stable for one week at 25°C, and the coupling procedure is highly reproducible.[80]

Molecules, such as antibodies that bind to cell surfaces, can be used to deliver cytotoxic drugs to selected cells. To be effective, the drug must usually be taken into the cells by endocytosis. Yemul et al. have reported that a T-cell line (CCRF-CEM) was effectively suppressed by liposomes carrying a photosensitizer and bearing the antibody OKT4 (anti-CD4).[81] A procedure has also been described whereby a photosensitizer, benzoporphyrin-derivative monoacid ring (BPD-MA), is covalently linked to a MAb in a manner that is reproducible, quantifiable, and retains both the biological activity of the antibody and the cytotoxicity of the photosensitizer. Preliminary steps involve linking BPD-MA to a modified polyvinyl alcohol (PVA) backbone, followed by conjugation to the antibody using heterobifunctional-linking technology.[82]

Specific binding to human ovarian adenocarcinomas of a drug-antibody conjugate (daunorubicin DNR-OC-125) made from a new analog (PIPP-DNR) of daunorubicin that chemically links the drug to monoclonal antibodies has been studied. Immunofluorescence data show that the DNR-OC-125 conjugate has high affinity and specificity for proliferating malignant cells from human ovarian tumors. The results further demonstrate that the DNR-OC-125 conjugate retains specific binding to CA-125 antigenic sites characteristic of the OC-125 monoclonal antibody moiety. The DNR-OC-125 conjugate selectively binds to CA-125 antigen-positive ovarian cancerous tissue in both cryostat and paraffin-embedded tissue sections. These results indicate that the OC-125 monoclonal antibody can serve as a cancer-targeting carrier for daunorubicin and its analogs.[83]

Bifunctional antibodies (BFA) and enzyme-conjugated antibodies (ECA) can be used to preferentially deliver a hapten or drug to tumor sites for diagnosis and therapy. The authors present here a simple pharmacokinetic model for the two systems by considering only two compartments: the plasma and tumor. The models predict that the longer the time delay between the BFA and hapten or between the ECA and prodrug injections, the higher the tumor-to-plasma-concentration ratio of the hapten drug.[78]

Cis-diamminedichloroplatinum (II) (Cis-Pt) has been complexed to a carboxymethyl dextran-avidin conjugate and targeted to biotin-monoclonal antibody 108(b-MAb108). This MAb recognizes the extracellular domain of the epidermal growth-factor receptor. The results presented in this preliminary investigation suggest that Pt-dex-Av is specifically removed from the circulation by b-MAb108 concentrated at the tumor site.[84]

Antibodies, because of their inherent specificity, appear to be ideal agents for recognizing and destroying malignant cells. However, MAbs, as currently constituted, still have certain inherent limitations. Transfectomas provide an approach to overcoming some of these limitations. Genetically engineered antibodies can be expressed following gene transfection into lymphoid cells.

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One of the major advantages of these antibodies is that one is not limited to naturally occurring antibodies. In particular, nonimmunoglobulin sequences can be joined to antibody sequences, thus creating multifunctional chimeric antibodies. In this way, growth factor binding capacity can be joined to a combining specificity, which may be useful in improving targeting therapy to malignant cells and delivering drugs into specific locales in the human body. The presence of the growth factor may also facilitate transcytosis of chimeric antibody across the blood–brain barrier using growth factor receptors. These novel chimeric antibodies may constitute a new family of immunotherapeutic molecules for cancer therapy.[76]

5-Fluorouridine (FUR), an antineoplastic agent, has been conjugated to the carbohydrate moiety of an anticarcinoembryonic antigen (CEA) MAb by using amino-dextran as the intermediate carrier. In the GW-39/nude mouse model, the conjugate remains efficient in targeting the human colonic tumor and possesses greater inhibitory growth effects on this subcutaneous tumor than free FUR or an irrelevant antibody conjugate. In addition, reduced host toxicity of the conjugate may permit its use in a high-dose therapy of this tumor system.[85]

Polyethylene glycol (PEG) modification of the MAb A7 has been found to enhance tumor localization. The F(ab')2 fragment of murine MAb A7 has been covalently bonded. PEG and the conjugate have been compared to the parent F(ab')2 fragment in *in vitro* and *in vivo* studies. PEG-conjugated antibody fragment was found to retain its antigen-binding activity in a competitive radioimmunoassay. The conjugate had a longer half-life and showed increased accumulation in tumors. Although the tumor:blood ratio for the parent F(ab')2 fragment was higher than that for the conjugate, it later showed a higher value than the whole MAb A7. Tissue:blood ratios were kept low with the conjugate, indicating that it was taken up in normal organs to a lesser extent as compared with the parent F(ab')2 fragment. These findings indicate that the PEG-conjugated F(ab')2 fragment may be a promising carrier for use in targeting cancer chemotherapy.[86]

The pharmacokinetics of a disulfide-linked conjugate of a murine monoclonal antibody A7 with neocarcinostatin (A7-NCS) has been studied following its intravenous administration to nude mice. The conjugate was removed from the blood circulation with a half-life of 12 hr, showing nearly the same kinetics as the free antibody. A7-NCS remained stable in the circulation and able to reach the target tumor without releasing significant free NCS.[87]

N-(2-Hydroxypropyl)methacrylamide (HMPA) copolymers have seen extensive development as lysosomotropic drug carriers. They can be used for site-specific drug delivery by incorporation of appropriate targeting groups. Specifically, they have been conjugated to antitumor MAbs murine IgG, antibody 872.3, and its F(ab') and F(ab')2 fragments. Conjugates were synthesized containing an average of 5 copolymer units (MW 20kD) per antibody molecule and achieved prolonged circulation in the bloodstream.[88]

Three novel prodrugs have been designed for use as anticancer agents. Each is a bifunctional alkylating agent that has been protected to form a

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relatively inactive prodrug. These prodrugs are designed to be activated to their corresponding alkylating agents at a tumor site by prior administration of an antitumor antibody conjugated to the bacterial enzyme carboxypeptidase G2 (CPG2) in a two-phase system called antibody-directed enzyme prodrug therapy (ADEPT). The potential of a tumor-localized bacterial enzyme to activate protected alkylating agents in order to eradicate an established human xenograft has been demonstrated.[89] Murine MAb A7 directed against human colon cancer has been chemically modified using methoxypolyethylene glycol (MPEG). A high substitution of PEG molecules on MAb A7 produces a progressive reduction in antibody-binding activity. The pharmacokinetic and immunological properties of MPEG-modified MAb A7 and the MPEG-modified F(ab′)2 fragment, which retained their antibody-binding activity, have been compared with parent MAb A7 and the F(ab′)2 fragment.

Blood clearance of MPEG-modified antibodies appears to be diminished by MPEG modification and fits a two-compartment model. Low MPEG-substituted MAb A7 showed less organ uptake in the liver and spleen and similar uptake in the lung and kidney when compared with the parent MAb A7. Both preparations exhibited less tissue:blood ratios in all respective organs as compared with parent antibodies. Tumor localization was enhanced by MPEG modification of the F(ab′)2 fragment, but not by MPEG modification for the whole MAb A7. Multiple intravenous administrations of MPEG-modified antibody to rabbits did not appear to elicit a measurable immune response. In conclusion, MPEG-modified antibodies are promising reagents as drug carriers to the target tumor.[90]

Two murine MAbs have been produced to losartan (DuP 753), a nonpeptide angiotensin II receptor antagonist. Using a solid-phase competitive enzyme-linked immunosorbent assay (ELISA), each antibody was examined for its ability to bind to a set of losartan analogs that differ structurally to varying degrees. Both antibodies distinguished fine structural changes in the analogs, particularly at the R5 position of the imidazole ring. No cross-reactivity toward either antibody was observed with the natural ligand angiotensin II, the peptide antagonist saralsin, or the AT2 selective nonpeptide antagonist.[91]

According to Goldenberg,[77] radionuclides, such as I and Tc, are used in antibody imaging. Radioactive antibodies have been found to be safe in over 10,000 patients studied worldwide. On a tumor-site basis, results from 60% to over 90% have been reported, with the highest accuracy rates occurring in MAbs labeled with ^{131}I, ^{123}I, and ^{99m}Tc. Other selected isotopes for antibody therapy are ^{90}yttrium, ^{186}rhenium, ^{188}rhenium, ^{67}copper, ^{211}astatine, and ^{125}iodine. Tumors as small as 0.5 cm have been identified using ^{99m}Tc-MAbs, especially with emission tomography, but resolution is usually in the range of 1.0 to 2.0 cm. Antibody imaging has revealed tumors missed by other methods, including computed tomography. Antibody imaging can be positive even before the antigen titer in the blood is elevated. Complexation with circulating antigen does not compromise antibody imaging.

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Cyclosporin and cyclophosphamide show a significant efficacy in most autoimmune diseases. However, their effects are dependent on continuous drug administration, which can present varied risks of toxicity, such as immunosuppression. Results recently obtained in animal models and discussed by Bach, particularly with anti-CD3 and anti-CD4 monoclonal antibodies, indicate that reestablishment of tolerance to self-antigens is a feasible goal.[92]

Experimental models of autoimmune diseases have demonstrated that such diseases can be prevented or treated by selectively interfering with the activation of any of the following cell types: antigen-presenting cells, autoreactive T cells, and regulatory T cells. Adorini et al. discuss these approaches to selective immunosuppression and examine how similar strategies may become applicable to the treatment of human autoimmune diseases.[93]

MAbs have potential as useful immunosuppressive agents. Short treatment courses with CD4/CD8 MAb can be used to guide the immune system of experimental animals to accept organ grafts and to arrest autoimmunity. This reprogramming has been reviewed by Wildmann and Cobbold, and is accompanied by potent T cell-dependent "infectious" regulatory mechanisms. A goal for therapeutic immunosuppression should be to understand and harness these innate immunoregulatory mechanisms.[94]

Hybridoma technology has enabled rodent MAbs to be created against human pathogens and cells. However, these have limited clinical utility. A strategy to develop effective antibodies for treating infectious disease, autoimmune disease, and cancer involves "humanizing" rodent antibodies. Humanized antibodies have improved pharmacokinetics, reduced immunogenicity, and have been used to clinical advantage.[95]

As mentioned previously, liposomes have a specific liquid-crystalline phase-transition temperature (Tc) at which they release an entrapped drug. Temperature-sensitive liposomes containing adriamycin (TS-Lip-ADM) have been made of dipalmitoylphosphatidylcholine, distearoylphosphatidylcholine, cholesterol, and adriamycin and conjugated with MAbs against human alphafetoprotein. When the liposomal suspension of ADM is immersed in a water bath, the release rate of ADM from TS-Lip-ADM-Ab also increases as the temperature increases from 34 to 42°C.[96]

Following the identification of antibodies as agents of immunity, it was hypothesized that individuals could be both protected against disease by the transfer of unmodified antibody (passive immunization), and cured of established disease by antibody armed with cytotoxic agents (immunotherapy). Although passive immunization has been practiced with great success for many years, successful tissue targeting by systemically delivered immunotoxins in humans has been documented in only a few cases. New modes of drug delivery, engineered for MAb-based products, may enable new applications of passive immunization and may provide improved tissue targeting for immunotherapy.[97]

Liposomes bearing surface-attached antibody (L-Ab) have been prepared to deliver dideoxyinosine triphosphate (ddITP) to human monocyte/macrophages. A mouse MAb (IgG[2a]) was modified using succinimidyl

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pyridyl dithiopropionate (SPDP) as a heterobifunctional reagent in order to conjugate the antibody to liposomes through a covalent (thioether) bond. Uptake of L-Ab by human monocyte/macrophages was measured as a function of time and compared to liposomes prepared with and without MPB-PE and free ddITP. It was concluded from these studies that the delivery of ddITP could be increased by surface-attached antibody.[74]

Dillman[107] reported on basic concepts and recent developments using monoclonal antibodies. Antibodies can serve as guiding and targeting systems for cytotoxic pharmaceutical products, such as radiolabeled antibodies,[107] for radioimmunodetection and radioimmunotherapy, immunotoxins, chemotherapy/antibody conjugates, cytokine/antibody conjugates, and immune cell/antibody conjugates. Interferon-alpha, interleukin-2 (IL-2), and various hematopoietic growth factors have important significance in biological therapy.

The advances in MAb production encouraged the initial concept of using cancer cell-specific "image bullets." A variety of agents (e.g., toxins, radionuclides, and chemotherapeutic agents) have been conjugated to mouse and human MAbs for selective delivery to neoplastic cells.[108]

Recently, MAbs that block activation of the EGFr and ErbB2 have been developed. These MAbs have shown promising preclinical activity, and "chimeric" and "humanized" MAbs have been produced in order to obviate the problem of host immune reactions.[109]

Trastuzumab, a humanized anti-ErbB2 MAb, was found active and was recently approved in combination with paclitaxel for the therapy of patients with metastatic ErbB2-overexpressing breast cancer. IMC-C225, a chimeric anti-EGFr MAb, demonstrated considerable activity when combined with radiation therapy and was found to reverse resistance to chemotherapy.[110]

Ozogamicin (Mylotarg) employs the antibody-targeted chemotherapy (ATC) strategy and has been approved by the U.S. Food and Drug Adminstration (FDA) for the treatment of CD33+ acute myeloid leukemia.[111]

Radiolabeled antitumor vascular endothelium monoclonal antibody (TES-23) was assessed in various tumor-bearing animals. This compound accumulated in KMT-17 fibrosarcoma. In meth-A fibrosarcoma, colon-26 adenocarcinoma in BALB/C mice and HT-1080 human tumor tissue in nude mice, radioactivities of 125I-TES-23 were up to 50 times higher than those of control antibody with insiginficant distribution to normal tissues. An immunoconjugate, composed of TES-23 and neocarzinostatin, was tested for its antitumor effect *in vivo*. TES-23-NCS, the immunoconjugate, caused a marked regression of tumor KMT-17 in rats and meth-A in mice.

Using hybridoma fusion, chemical characterization, or molecular biology technology, antibodies with dual specificity can be constructed. The so-called biospecific antibodies (BsAbs) have been used to redirect the cytolytic activity of a variety of immune-effector cells, such as cytotoxic T lymphocytes, natural killer cells, neutrophils, and monocytes/macrophages to tumor cells. Local administration of BsAbs, either alone or in combination with analogous effector cells, has been found highly effective in eradicating tumor cells.[112]

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Clinical use of monoclonal antibodies that were produced against the cytokines and adhesion molecules, such as IL-1, IL-6, IG-6R, TNF-alpha, and CD4 molecules, was found effective for the treatment of rheumatoid arthritis. However, these therapeutic agents were also found to exhibit several disadvantages, such as transient efficacy and undesirable side effects.[113]

Takayanagi et al.[114] reported on the "immunogene" system for the targeted delivery of therapeutic genes. In their study, the immunogene system utilizes the EGF receptor-mediated endocytosis. The Fab fragment of MAb B4G7 against human EGF factor was conjugated with polyglycine to form a "Fab-immunoporter," which forms an affinity complex with DNA. The transfection efficiency of Fab immunogene was approximately tenfold higher than the lipofectin. Gene transfer of HSV-tk gene into A431 tumor cells with Fab-immunoporter was successful, and the subsequent treatment with ganciclovir induced remarkable side effects, conferring thousandfold higher drug sensitivity. According to these authors, their data demonstrated that the immunogene system could be useful as a gene transfer vehicle targeting the EGF receptor hyperproducing tumor cells.

Over the years, delivery of monoclonal antibodies has developed some problems, especially in their pharmacokinetic aspects. These include slow elimination of monoclonal antibodies from the blood and poor vascular permeability, low and heterogeneous tumor uptake, cross-reactivity with normal tissues, metabolism of monoclonal antibody conjugates, and immunogenicity of murine forms in humans. Progress has been made in solving these problems (e.g., tumor retention of antibody conjugates may be improved by inhibition of metabolism), and by using stable-linkage chemistry, normal tissue retention may be decreased through the use of metabolizable chemical linkages inserted between the antibody and the conjugated moiety.[115]

V. Conclusion and basis for future trends

It is becoming apparent that work on the use of MAbs for drug targeting is progressing steadily toward increased clinical use.[98] The applications developed at present have been primarily in cancer chemotherapy, where the greatest need arises for site-specific drug delivery. MAbs are also increasingly used in heart disease, multiple sclerosis, disorders of the immunological defense system, and viral, bacterial, and rickettsial infections. Since each MAb is directed against a single determinant, it attains a finer, more specific recognition of its antigen than conventional antibodies. Investigators have been using MAbs as exquisitely sensitive probes to guide drugs to target cells or organs. As more and more MAbs directed against normal and tumor cells are generated, it will be possible to have a spectrum of these antibodies. Each of these will identify a distinct molecular determinant on the cell surface to better define the stages of lymphoid cell differentiation, for example, as well as a more precise and reliable classification of cell malignancies and immunodeficiencies in humans.[99–101]

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MAbs are also being standardized as pure reagents, thereby replacing conventionally made tissue-typing reagents. These MAbs have undoubtedly helped to develop more rational therapeutic strategies for the treatment and prognosis of certain diseases. MAbs by themselves or in combination with surgery have a strong potential for immunotherapy of serum hepatitis, leukemias, etc.

MAbs have opened up new immunodiagnostic markets for identifying various leukemic and lymphoma cell populations, characterization of isoenzymes, hemoglobins, α-1-antitrypsins, lymphokines, hormones, hepatitis-associated antigens, carcinoembryonic antigens, assays for therapeutic drug and drug-abuse monitoring, and specific protein immunoassays. Since they are well-defined chemical reagents, MAbs have great potential for practical applications either by their direct use or through their utilization in drug development.

The production of human monoclonals of predefined human-target site specificity by hybridoma technology has achieved limited success. As anticipated, the immunogenicity of human target structures is different in humans than in other species, and although *in vitro* immunization techniques can work, they have so far failed to produce useful antitumor human monoclonals. It is likely that in the future, modern techniques of molecular gene cloning will be used to produce the required structures. Experiments have been described demonstrating the feasibility of using recombinant DNA technology to produce chimeric antibody molecules in which the antigen-combining site is derived from a mouse myeloma and the constant region of the molecule is derived from human immunoglobulin.[102–104]

Improvements in the supply of immune lymphocytes are feasible, both for immunization protocols and lymphocyte-selection procedures. Of clinical importance are the advances made in *in vitro* immunization procedures. However, a number of factors require extensive investigation before *in vitro* immunization can be fully exploited. These include the source of lymphoid cells, the dose and form of immunogen, the influence of mitogens and adjuvants, the value of growth and differentiation factors, and optimum culture time. The most widely used immortalization procedures have been EBV, transformation and cell fusion, or a combination of these procedures. However, it is possible that these procedures are still far from optimal. Meanwhile, considerable effort has been directed toward improving the levels of antibody secreted by human cell lines and ensuring that high levels of secretion are sustained. This observation may partly be due to improvements in immunization, selection, and immortalization. The most obvious approach has been to frequently enrich antibody-secreting cell lines and pursue a rigorous cloning policy.

A less conventional approach to human MAb production has been the application of gene cloning strategies, but the results have nonetheless been encouraging. So far, complete immunoglobulin molecules have been produced using conventional recombinant procedures employing vectors. Another less sophisticated strategy has been to transfect the antibody-secret-

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ing cells. Other advances in this technique for producing antibodies have also indicated considerable potential, and more research and development work in this area should prove valuable.[75,78,105,106]

As summarized by Rowland,[12] it is the specificity conferred by antibody molecules that is now exploited for novel methods of therapy. Without doubt, advances in cellular and molecular biology need to be prudently applied to this area in the years ahead to procure useful site-specific drug delivery systems.

References

1. Ehrlich, P., A general review of the recent work in immunity. In *Collected Papers of Paul Ehrlich*, Vol. 2, *Immunology and Cancer Research 1956,* Pergamon Press, London, 1990, 442.
2. Larson, S.M., Brown, J.P., Wright, P.W., Carrasquillo, J.A., Hellstrom, I., and Hellstrom, K.E., Imaging of melanoma with I-labeled monoclonal antibodies, *J. Nuclear Med.*, 24, 123–129, 1983.
3. Zurawski, V.R., Haber, E., and Black, P.H., Production of antibody to tetanus toxoid by continuous human lymphoblastoid cell lines, *Science*, 199, 1439, 1978.
4. Trout, A., Increased selectivity of drugs by linking to carriers, *Eur. J. Cancer*, 14, 105, 1978.
5. Steinitz, M., Klein, G., Koskimies, S., and Makela, O., EB virus-induced B-lymphocyte cell lines producing specific antibody, *Nature*, 269, 420, 1977.
6. Marguilies, D.H., Kuehl, W.M., and Schraff, M.D., Somatic cell hybridization of mouse myeloma cells, *Cell*, 8, 405, 1976.
7. Kennett, R.H., Denis, K.A., Tung, A.S., and Klinman, N.R., Hybrid plasmacytoma production fusions with adult spleen cells, monoclonal spleen fragments, neonatal spleen cells and human spleen cells, *Curr. Top. Microbiol. Immunol.*, 81, 77, 1978.
8. Kearney, J.F., Redbruck, A., Liesegaug, B., and Rajewskey, K., A new mouse myeloma cell line that has lost immunoglobulin expression but permits the construction of antibody-secreting hybrid cell lines, *J. Immunol.*, 123, 1548, 1979.
9. Birch, J.R., Cells sell, *Chemtech*, 17(6), 378–381, 1987.
10. Schen, W., Chemical aspects of monoclonal antibodies in targeted drug delivery, *Pharm. Tech.*, 32(12), 1988.
11. Rowland, G.F., Davies, D.A.L., O'Neill, G.I., Newman, C.E., and Ford, C.H.J., Specific cancer therapy by drugs synergising with or attached to tumor-specific antibodies: experimental background and clinical results. In *Immunotherapy of Malignant Diseases*, Rainer, H., Ed., Schattauer-Verlag, Stuttgart, 1977, 316–322.
12. Rowland, G.F., Monoclonal antibodies as carriers for drug delivery systems. In *Fundamentals and Techniques*, Johnson, P. and Lloyd-Jones, J.G., Eds., VCH Publishers, Chichester, 1987, 81–94.
13. Brown, J.P., Woodbury, R.G., Hart, C.E., Hellstrom, I., and Hellstrom, K.E., Quantitative analysis of melanoma-associated antigen p97 in normal and neoplastic tissues, *Proc. Nat. Acad. Sci., USA*, 78, 539–543, 1981.

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14. Hellstrom, K.E. and Hellstrom, I., Monoclonal antimelanoma antibodies and their possible clinical use. In *Monoclonal Antibodies for Cancer Detection and Therapy*, Baldwin, R.W. and Byerrs, V.S., Eds., Academic Press, London, 1985, 17–51.
15. Mathe, G., Loc, T.B., and Bernard, J., Effet sur la leucemie L1201 de la souris d'une combinaison par diazotation d'A-methopterine et de gamma-globulins de hamsters porteur de cette leucemie par heterogreffe, *Comptes Rendus*, 246, 1626–1628, 1958.
16. Ghose, T., Norwell, S., Guclu, A., Cameron, D., Bodrutha, A., and MacDonald, A.S., Immunotherapy of cancer with chlorambucil-carrying antibody, *Br. Med. J.*, iii, 495, 1972.
17. Hurwitz, E., Maron, R., Bernstein, A., Wilcheck, M., Sela, M., and Arnon, R., The effect *in vivo* of chemotherapeutic drug-antibody conjugates in two murine experimental tumor systems, *Int. J. Cancer*, 21, 747–755, 1978.
18. Hurwitz, E., Maron, R., Wilcheck, M., Arnon, R., and Sela, M., The covalent binding of daunomycin and adriamycin to antibodies with retention of both drug and antibody activities, *Cancer Research*, 35, 1175–1181, 1975.
19. Rowland, G.F., O'Neill, G.I., and Davies, D.A.L., Suppression of tumor growth in mice by a drug-antibody conjugate using a novel approach to linkage, *Nature*, 255, 487–488, 1975.
20. Kadin, S.B. and Otterness, I.G., Antibodies as drug carriers and toxicity-reversal agents, *Ann. Rep. Med. Chem.*, 15, 233–244, 1980.
21. Upselacis, J. and Hinman, L., Chemical modification of antibodies for cancer chemotherapy, *Ann. Rep. Med. Chem.*, 23, 151–160, 1988.
22. Kohler, G. and Milstein, C., Continuous cultures of fused cells secreting antibody of predefined specificity, *Nature*, 256, 495, 1975.
23. Gilliland, D.G., Steplewski, Z., Collier, R.J., Mitchell, K.F., Chang, T.H., and Koprowski, H., Antibody-directed cytotoxic agents: Use of monoclonal antibody to direct the action of toxin A chains to colorectal carcinoma cells, *Proc. Natl. Acad. Sci. USA*, 77, 4539, 1980.
24. Sickle-Santanello, B.J. et al., Radioimmunoguided surgery using monoclonal antibody B72.3 in colorectal tumors, *Dis. Col. Rectum*, 30, 761–764, 1987.
25. Greiner, J.W. et al., Recombinant interferon enhances monoclonal antibody-targeting of carcinoma lesions *in vivo*, *Science*, 235(4791), 895–898, 1987.
26. Rowland, G.F., The use of antibodies and polymer conjugates in drug targeting and synergy. In *Target Drugs*, Goldberg, E., Ed., John Wiley & Sons, New York, 1983, 57–72.
27. Brown, J.P., Noshiyama, K., Hellstrom, I., and Hellstrom, K.E., Structural characterization of human melanoma-associated antigen p97 with monoclonal antibodies, *J. Immunol.*, 127, 539–546, 1981.
28. Jarvis, A.P. Jr., Producing monoclonal antibodies for clinical investigations. In The Latest Developments in Drug Delivery Systems, Conference Proceedings, *Pharm. Tech.*, 11, 48–53, 1987.
29. Holyoke, E.D. and Petrelli, N.J., Tumor markers and monoclonal antibodies: An update, *Medical Times*, 57–63, 1987.
30. Olsson, L. and Kaplan, H., Human-human hybridomas producing monoclonal antibodies of predefined antigenic specificity, *Proc. Nat. Acad. Sci. USA*, 77, 5429, 1980.
31. Kennett, R.H., McKearn, T.J., and Bechtol K.B., Eds., *Monoclonal Antibodies*, Plenum Press, Inc., New York, 1980.

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32. Cortesis, G.P. and Proby, C.M., Airlift bioreactors for production of monoclonal antibodies, *Biopharm*, Nov. 1987, 30.
33. Lebherz III, W.B., Batch production of monoclonal antibody by large-scale suspension culture, *Biopharm*, Feb. 1988, 22.
34. Familletti, P.G., Gel-immobilized cell culture for monoclonal antibody production, *Biopharm*, Nov. 1987, 48.
35. Koprowski, G., Steplewski, Z., Herlyn, D., and Herlyn, M., Study of antibodies against human melanoma produced by somatic cell hybrids, *Proc. Nat. Acad. Sci. USA*, 75, 3405, 1978.
36. Carrel, S., Accolla, R.S., Gross, N. and Mach, J.P., Human melanoma-associated antigens identified by monoclonal antibodies. In *Monoclonal S4Antibodies and T-Cell Hybridoma Perspectives and Technological Advances*, Hammerling, G.J., Hammerling, Y., and Kearney, J.F., Eds., Elsevier, Amsterdam, 1981, 174.
37. Imai, K., Wilson, B.S., Kay, N.E. and Ferrone, S., Monoclonal antibodies to human melanoma cells comparison of serological results of several laboratories and molecular profile of melanoma-associated antigens. In *Monoclonal S4Antibodies and T-Cell Hybridoma Perspectives and Technological Advances*, Hammerling, G.J., Hammerling, Y., and Kearney, J.F., Eds., Elsevier, Amsterdam, 1981, 183.
38. Herlyn, M., Steplewski, Z., Herlyn, D., and Koprowski, H., Colorectal carcinoma-specific antigen detection by means of monoclonal antibodies, *Proc. Nat. Acad. Sci. USA*, 76, 1438, 1979.
39. Hellstrom, I., Hellstrom, K.E., Brown, J.P., and Woodbury, R.G., Antigens of human tumors, particularly melanomas, as studied with the monoclonal antibody technique. In *Monoclonal Antibodies and T-Cell Hybridoma Perspectives and Technological Advances*, Hammerling, G.J., Hammerling, Y. and Kearney, J.F., Eds., Elsevier, Amsterdam, 1981, 191.
40. Kennett, R.H. and Gilbert, F., Hybrid myelomas producing antibodies against a human neuroblastoma antigen present on fetal brain, *Science*, 203, 1120, 1979.
41. Schlom, J., Monoclonal antibodies in cancer therapy: the present and the future, *Biopharm*, Sept. 1988, 44–48. (Also see *Pharm. Tech.*, Sept. 1988, 56–60.)
42. Schlom, J. and Weeks, M.O., Potential clinical utility of monoclonal antibodies. In *The Management of Human Carcinomas in Important Advances in Oncology,* DeVita, V., Hellman, S., and Rosenberg, S., Eds., J.B. Lippincott, Philadelphia, 1984, 170–192.
43. Order, S.E., Analysis, results and future prospective of the therapeutic use of radiolabelled antibody in cancer therapy. In *Monoclonal Antibodies for Cancer Detection and Therapy,* Baldwin R.W. and Byers, V.S., Eds., Academic Press, London, 1985, 304–306.
44. Widder, K.J., Senyei, A.E., and Ranney, D.F., Magnetically responsive microspheres and other carriers for the biophysical targeting of antitumor agents, *Adv. Pharmacol. Chemotherapy,* 16, 213, 1979.
45. O'Neill, G.J., The use of antibodies as drug carriers. In *Drug Carriers in Biology and Medicine*, Gregoriadis, G., Ed., Academic Press, Inc., London, 1979, 23–41.
46. Gallego, J., Price, M.R., and Baldwin, R.W., Preparation of four daunomycin-monoclonal antibody 791T/36 conjugates with anti-tumor activity, *Int. J. Cancer*, 33, 737–744, 1984.
47. Rowland, G.F., Use of antibodies to target drugs to tumor cells, *Clinics in Allergy and Immunology*, 8, 2, 235–257, 1983.

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48. Arnon, R. and Hurwitz, E., Monoclonal antibodies as carriers for immuno-targeting of drugs. In *Monoclonal Antibodies for Cancer Detection and Therapy*, Baldwin R.W. and Byers, V.S., Eds., Academic Press, London, 1985, 367–383.
49. Hockey, M.S., Stokes, H.J., Thompson, H., Woodhouse, C.S., MacDonald, F., Fielding, J.W.I., and Ford, C.H.I., Carcinoembryonic antigen (CEA) expression and heterogeneity in primary and autologous metastatic gastric tumors demonstrated by a monoclonal antibody, *Br. J. Cancer*, 49, 192–233, 1984.
50. Philpott, G.W., Grass, E.H., and Parker, C.W., Affinity cytotoxicity with an alcohol dehydrogenase-antibody conjugate and allyl alcohol, *Cancer Res*., 39, 2084, 1979.
51. Rowland, G.F., Corvalan, J.R.F., Axton, C.A., Gore, V.A., Marsden, C.H., Smith, W., and Simmonds, R.G., Suppression of growth of a human colorectal tumor in nude mice by vindesine-monoclonal anti-CEA conjugates, *Protides Biol. Fluids*, 31, 783–786, 1984.
52. Rowland G.F., Axton, C.A., Baldwin, R.W., Brown, J.P., et al., Antitumor properties of vindesine-monoclonal antibody conjugates, *Cancer Immunol. Immunother.*, 19, 1–7, 1988.
53. Rowland, G.F., Simmonds, R.G., Gore, V.A., Marsden, C.H., and Smith, W., Drug localization and growth-inhibition studies of vindesine-monoclonal anti-CEA conjugates in a human tumor xenograft, *Cancer Immunol. Immunother.*, 21, 183–187, 1986.
54. Levy, R. and Dilley, J., Rescue of immunoglobulin secretion from human neoplastic lymphoid cells by somatic cell hybridization, *Proc. Nat. Acad. Sci. USA*, 75, 2411, 1978.
55. Vaughan, A.T.M., Bradwell, A.R., Dykes, P.W. and Anderson, P., Illusions of tumor killing using radiolabelled antibodies, *Lancet*, 1492, 1986.
56. Ford, C.H.J., Newman, C.E., Johnson, J.R., Woodhouse, C.S., et al., Localization and toxicity study of a vindesine-anti-CEA conjugate in patients with advanced cancer, *Br. J. Cancer*, 47, 35–42, 1983.
57. Dykes, P.W., Hine, K.G., Bradwell, A.R., Blackburn, J.C., et al., Localization of tumor deposits by external scanning after injection of radiolabelled anti-carcinoembryonic antigen, *Br. Med. J*., 280, 220–222, 1980.
58. Jansen, F.K., Blythman, H.E., Carrierre, D., Casellas, P., et al., Assembly and activity of conjugates between monoclonal antibodies and the toxic subunit of ricin (immunotoxins) in monoclonal antibodies, *Proc. Nat. Acad. Sci. USA*, 76, 229, 1979.
59. Blythman, H.E., Casellas, P., Gross, O., Gross, P., et al., Immunotoxins: Hybrid molecules of monoclonal antibodies and a toxin subunit specifically kill tumor cells, *Nature* (London), 290, 145, 1981.
60. McKearn, T.J., Weiss, A., Stuart, F.F., and Fitch, F.W., Eds., Selective suppression of humoral and cell-mediated immune responses to rat alloantibodies by monoclonal antibodies produced by hybridoma cell lines, *Transplant Proc*., 11, 932, 1979.
61. Melchers, F., Potter, M., and Warnen, N.L., Eds., Lymphocyte hybridomas, *Curr. Top. Microbiol. Immunol*., 81, 246, 1978.
62. D'Eustachio, P. and Ruddle, F.H., *Current Topics in Developmental Biology*, Vol. 14, Frieldlander, M., Ed., Academic Press, New York, 1980, 59.
63. Cosimi, A.B., Burton, R.C., Kung, P.C., Colvin, R., et al., Evaluation in primate renal allograft recipients of monoclonal antibody to human T-cell, *Transplant Proc*., 13, 499, 1981.

© 2004 by CRC Press LLC

64. Potocnjak, P., Yoshida, N., Nussenzweig, R.S., and Nussenzweig, V., Monovalent fragments (Fab) of monoclonal antibodies to a sporozoite surface antigen (Pb44) protect mice against malarial infection, *J. Exp. Med*., 151, 1564, 1980.
65. Witkor, T.J. and Koprowski, H., Monoclonal antibodies against rabies virus produced by somatic cell hybridization: Detection of antigenic variants, *Proc. Nat. Acad. Sci. USA,* 75, 3938, 1978.
66. Bernstein, I.D., Tam, M.R., and Nowinski, R.C., Mouse leukemia therapy with monoclonal antibodies against a thymus differentiation antigen, *Science*, 207, 68, 1980.
67. Tung, A.S., Monoclonal antibodies, *Ann. Rep. Med. Chem*., 16, 243–255, 1981.
68. Ferrone, S. and Dietrich, M.P., Eds., *Handbook of Monoclonal Antibodies: Applications in Biology and Medicine*, 1985, 477.
69. Perrin, C.H., Ramirez, E., Lambert, P.H., and Miescher, P.A., Inhibition of *P. falciparum* growth in human erythrocytes by monoclonal antibodies, *Nature* (London), 289, 301, 1981.
70. Freeman, R.R., Trejdosiewicz, A.J., and Cross, G.A.M., Protective monoclonal antibodies recognizing stage-specific merozoite antigens of a rodent malaria parasite, *Nature* (London), 284, 366, 1980.
71. Verwaere, C., Grzch, J.M., Bazin, H., Capron, M., and Capron, A., Production d'anticorps monoclonaux anti Schistosoma monsoni Etude preliminaire de Leurs activites biologiques, *CR Acad. Sci. Ser. D*., 289, 725, 1979.
72. Mitchell, C.F., Cruise, K.M., Chapman, C.B., Anders, R.F., and Howard, M.C., Hybridoma antibody immunoassays for the detection of parasitic infection: Development of a model system using a larval cestode infection in mice, *Aust. J. Exp. Biol. Med. Sci*., 57, 287, 1979.
73. Pharmaprojects, 1993.
74. Betageri, G.V., Jenkins, S.A., and Ravis, W.R., Drug delivery using antibody-liposome conjugates, *Drug Dev. Ind. Pharm*., 19/16, 2109–2116, 1993.
75. Goding, J.W., *Monoclonal Antibodies: Principles and Practice*, Academic Press, San Diego, CA, 1986, 315.
76. Shin, S.U., Chimeric antibody: potential applications for drug delivery and immunotherapy, *Biotherapy*, 3, 43–53, 1991.
77. Goldenberg, D.M, New developments in monoclonal antibodies for cancer detection and therapy, *CA Cancer J. Clin.* (U.S.), 44, 43–64, 1994.
78. Oates, K.K., Therapeutic and drug delivery application of monoclonal antibodies, *Targeted Diagn. Ther*., 3, 99–118, 1990.
79. Wolozin, B.L., Pruchnicki, A., Dickson, D.W., and Davies, P., A neuronal antigen in the brains of Alzheimer patients, *Science*, 232(4750), 648–650, 1986.
80. Page, M., Thibeault, D., Noel, C., and Dumas, L., Coupling a preactivated daunorubicin to antibody, *Anticancer Res*., 10, 353–357, 1990.
81. Yemul, S., Berger, C., Katz, M. Estabrook, A., et al., Phototoxic liposomes coupled to an antibody, *Cancer Immunol. Immunother*., 30, 317–322, 1990.
82. Jiang, F.N., Jiang, S., Liu, D., Richter, A., and Levy, J.G., Development of technology for linking photosensitizers, *J. Immunol. Methods*, 134, 139–149, 1990.
83. Dezso, B., Torok, I., Rosik, L.O., and Sweet, F., Human ovarian cancers specifically bind daunorubicin-OC-125 conjugate, *Gynecol. Oncol*., 39, 60–64, 1990.
84. Schechter, B., Arnon, R., Wilchek, M., Schlessinger, J., et al., Indirect immunotargeting of cis-Pt to human epidermoid carcinoma KB using the avidin-biotin system, *Int. J. Cancer*, 48, 167–172, 1991.

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85. Shih, L.B., Xuan, H., Sharkey, R.M., and Goldenberg, D.M., A fluorouridine-anti-CEA immunoconjugate is therapeutically effective in a human colonic xenograft model, *Int. J. Cancer*, 46, 1101–1106, 1990.
86. Kitamura, K., Takahashi, T., Takahashi, K., Yamagucbi, T., et al., Polyethylene glycol modification of the monoclonal antibody A7 enhances its tumor localization, *Biochem. Biophys. Res. Commun.*, 171, 1387–1394, 1990.
87. Kitamura, K., Takahashi, T., Noguchi, A., Tsurumi, H., et al., Pharmacokinetic analysis of the monoclonal antibody A7 neo-carzinostatin conjugate administered to nude mice, *Tohoku J. Exp. Med.*, 164, 203–211, 1991.
88. Seymour, L.W., Flanagan, P.A., al-Shamkhani, A., Subr, V., et al., Synthetic polymers conjugated to monoclonal antibodies: Vehicles for tumor-targeted drug delivery, *Sel. Cancer Ther.*, 7, 59–73, 1991.
89. Springer, C.J., Bagshawe, K.D., Sharma, S.K., Searle, F., et al., Ablation of human choriocarcinoma xenografts in nude mice by antibody-directed enzyme pro-drug therapy with three novel compounds, *Eur. J. Cancer*, 27, 1361–1366, 1991.
90. Kitamura, K., Takahashi, T., Yamaguchi, T., Noguchi, A., et al., Chemical engineering of the monoclonal antibody A7 by polyethylele glycol, *Cancer Res.*, 51, 4310–4315, 1991.
91. Reilly, T.M., Christ, D.D., Duncia, J.V, Pierce, S.K., and Timmermans, P.B, Monoclonal antibodies to the nonpeptide angiotensin II receptor antagonist, losartan, *Eur. J. Pharmacol*. (Netherlands), 226, 179–182, 1992.
92. Bach, J., Immunosuppressive therapy of autoimmune diseases, *Trends Pharmacol. Sci*. (U.K.), 14, 213–216, 1993.
93. Adorini, L., Guery, J., Rodriguez-Tarduchy, G., and Trembleau, S., Selective immunosuppression, *Trends Pharmacol. Sci.* (U.K.), 14, 178–182, 1993.
94. Wildmann, H. and Cobbold, S., The use of monoclonal antibodies to achieve immunological tolerance, *Trends Pharmacol. Sci.* (U.K.), 14, 143–148, 1993.
95. Winter, G. and Harris, W., Humanized antibodies, *Trends Pharmacol. Sci.* (U.K.), 14, 139–143, 1993.
96. Shibata, S., Kumai, K., Takahashi, T., Murayama, Y., et al., Targeting cancer chemotherapy using temperature-sensitive liposomes containing adriamycin conjugated with monoclonal antibodies, *Gan To Kagaku Ryoho* (Japan), 19 (Suppl. 10), 1671–1674, 1992.
97. Saltzman, W.M., Antibodies for treating and preventing disease: the potential role of polymeric controlled release, *Crit. Rev. Ther. Drug Carrier Syst*. (U.S.), 10, 111–142, 1993.
98. Brown, J., Ed., *Human Monoclonal Antibodies: Current Techniques and Future Perspectives*, IRL, Oxford, 1987.
99. Cohn, M., Langman, R., and Geckler, W. In *Progress in Immunology*, Vol. 4, Fougereau, M., Dausset, J., Eds., Academic Press, New York, 1980, 153.
100. Dennis, K., Kennett, R.H., Klinman, N.R., Molinaro, C., and Sherman. L. In *Monoclonal Antibodies*, Kennett, R.H., McKearn, T.J., and Bechtol, K.B., Eds., Plenum Press, New York, 1980, 49.
101. Solomon, E. and Jones, E.A. In *Monoclonal Antibodies*, Kennett, R.H., McKearn, T.J., and Bechtol, K.B., Eds., Plenum Press, New York, 1980, 75.
102. Handman, E. and Remington, J.S., Monoclonal antibodies diagnostic and therapeutic use. In *Tumor and Transplantation*, Chatterjee, S.N., Ed., PSG Publishing Co., Littleton, MA, 1985.

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103. Pinder, M. and Hewerr, R.S., Monoclonal antibodies detect antigenic diversity in *Theileria parva* parasites, *J. Immunol*., 124, 1000, 1980.
104. Handman, E. and Remington, J.S., Serological and immunochemical characterization of monoclonal antibodies of *Toxoplasma gondii*, *Immunology*, 40, 4, 1980.
105. Begent, R.H., Pedley, R.B., and Begent, J., Monoclonal antibody administration, current clinical pharmacokinetic status and future trends, *Clin. Pharmacokinet*. (New Zealand), 23, 85–89, 1992.
106. Liddell, J.E. and Cryer, A. In *A Practical Guide to Monoclonal Antibodies*, John Wiley & Sons, Chichester, 1991, 206.
107. Dillman, R.O., Monoclonal antibodies in the treatment of malignancy: basic concepts and recent developments, *Cancer Invest*., 19, 833–841, 2002.
108. Bodey, B., Genetically engineered antibodies for direct antineoplastic treatment and systemic delivery of various therapeutic agents to cancer cells, *Expert Opinons Biol. Ther*., 1, 603–617, 2002.
109. Mendelsonh, J. and Baselga, J., The EGF receptor family as targets for cancer therapy, *Oncogene*, 19, 6550–6565, 2000.
110. Williams, J.P. and Handler, H.L., Antibody-targeted chemotherapy for the treatment of relapsed acute myeloid leukemia, *Am. J. Manag. Care*, 6(Suppl. 18), S975–S985, 2000.
111. Tsunoda, S., Tsutsumi, Y., et al., Targeting therapy using a monoclonal antibody against tumor vascular endothelium, *Yakugaku Zasshi*, 120, 256–264, 2000.
112. Molema, G., Kroesen, B.J., et al., The use of bispecific antibodies in tumor cell- and tumor vasculature-directed immunotherapy, *J. Control Rel*., 64, 229–239, 2000.
113. Abe, T. and Takeuchi, T., Monoclonal antibodies as an immunotherapy of rheumatoid arthritis, *Nippon Rinsho*, 56, 776–781, 1998.
114. Takayanagi, A., Chen, J., et al., Targeting delivery of therapeutic genes using monoclonal antibody; immunogene approach, *Nippon Rinsho*, 56, 731–736, 1998.
115. Reilly, R.M., Sandhu, J., et al., Problems of delivery of monoclonal antibodies, pharmaceutical and pharmacokinetic solutions, *Clin Pharmacokin*., 28, 126–42, 1995.

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