Targeted Therapy of Cancer: New Prospects for Antibodies and Immunoconjugates[1]

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ABSTRACT Immunotherapy of cancer has been explored for over a century, but it is only in the last decade that various antibody-based products have been introduced into the management of patients with diverse cancers. At present, this is one of the most active areas of clinical research, with eight therapeutic products already approved in oncology. Antibodies against tumor-associated markers have been a part of medical practice in immunohistology and in vitro immunoassays for several decades, have even been used as radioconjugates in diagnostic imaging, and are now becoming increasingly recognized as important biological agents for the detection and treatment of cancer. Molecular engineering has improved the prospects for such antibody-based therapeutics, resulting in different constructs and humanized/human antibodies that can be administered frequently. Consequently, a renewed interest in the development of antibodies conjugated with radionuclides, drugs, and toxins has emerged. We review how antibodies and immunoconjugates have influenced cancer detection and therapy, and also describe promising new developments and challenges for broader applications. (*CA Cancer J Clin 2006;56:226–243.*) © American Cancer Society, Inc., 2006.*

INTRODUCTION

The search for a mechanism to target diseases selectively was first realized when resistance to infectious disease could be transferred from one animal to another through their serum, a process known as passive serotherapy.[1] Five years later, in 1895, Hericourt and Richet immunized dogs with a human sarcoma and then transferred the serum to patients.[2] This anticipated the "magic bullet" concept of Paul Ehrlich in 1908, that "toxins" could be targeted to cancer and other diseases.[3] Another half-century passed before antibodies were identified as the substance in serum responsible for these effects.

Despite being potent immune system instigators for killing infectious agents, clinical research initially focused on immunoconjugates prepared with radionuclides, drugs, or toxins, since unconjugated or "naked" antibodies had little therapeutic benefit in oncology compared with the immunoconjugates. Early immunotherapy trials failed to show substantial responses,[4–6] but antibodies against carcinoembryonic antigen (CEA) could selectively target and disclose sites of CEA-expressing cancers in patients, and also deliver cytotoxic radioactivity in human colonic cancer xenografts having CEA.[7,8] Thereafter, DeNardo, et al.[9] reported responses in lymphoma patients to radiolabeled antibodies, and soon others confirmed that radiolabeled antibodies had antitumor activity in non-Hodgkin lymphoma (NHL), but there was also early evidence that the naked antibodies themselves might be effective.[10–12] It was during this same period that rituximab (Rituxan, Genentech, and biogen idec), an anti-CD20 IgG, became of interest as a therapeutic for NHL without being radiolabeled.[13] The experience and subsequent introduction of rituximab into the treatment of NHL can be credited for the expanded interest in unconjugated antibodies for cancer therapy.

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Antibodies (eg, IgG, which is the most commonly used immunoglobulin form, Figure 1) are unique proteins with dual functionality. All naturally occurring antibodies are multivalent, with IgG having two binding 'arms.' Antigen-binding specificity is encoded by three *complementarity-determining regions* (CDRs), while the *Fc-region* is responsible for binding to serum proteins (eg, complement) or cells. An antibody itself usually is not responsible for killing target cells, but instead marks the cells that other components or effector cells of the body's immune system should attack, or it can initiate signaling mechanisms in the targeted cell that leads to the cell's self-destruction (Figure 2). The former two attack mechanisms are referred to as antibody-dependent *complement-mediated cytotoxicity* (CMC) and *antibody-dependent cellular cytotoxicity* (ADCC). ADCC involves the recognition of the antibody by immune cells that engage the antibody-marked cells and either through their direct action, or through the recruitment of other cell types, lead to the tagged-cell's death. CMC is a process where a cascade of different complement proteins become activated, usually when several IgGs are in close proximity to each other, either with one direct outcome being cell lysis, or one indirect outcome being attracting other immune cells to this location for effector cell function.

Antibodies, when bound to key substances found on the cell surface, also can induce cells to undergo *programmed cell death*, or *apoptosis* (Figure 2). For example, if rituximab binds to two CD20 molecules, this triggers signals inside the cell that can induce apoptosis.[14] If rituximab is cross-linked by other antiantibodies, the apoptotic signal is intensified.[15] This cross-linking could also occur when the antibody is bound by another immune cell through its Fc-gamma receptors (FcγR). Other antibodies, such as trastuzumab (anti-HER2/*neu*; Herceptin, Genentech) and cetuximab (anti-epidermal growth factor receptor, EGFR; Erbitux, ImClone Systems and Bristol-Myers Squibb) also have the ability to inhibit cell proliferation.[16–18] Because cells frequently have alternative pathways for critical functions, interrupting a single signaling pathway alone might not be sufficient to ensure cell death. From this perspective, it is not surprising that antibodies are often best used in combination with chemotherapy and radiation therapy to augment their antitumor effects.[19–21]

Bevacizumab (Avastin, Genentech) is yet another example of how antibodies can be used therapeutically. This antibody binds to vascular endothelial growth factor (VEGF) that is made by tumor cells to promote vessel formation, thereby preventing it from interacting with endothelial cells to form new blood vessels (Figure 2).[22] Antibodies can also be used to modulate immune response. Antibodies to the cytotoxic T-lymphocyte associated antigen-4 (CTLA-4) stimulate T-cell immune responses by blocking the inhibitory effects of CTLA-4, which can enhance tumor rejection.[23] However, release of this innate inhibitory mechanism can also increase the risk of autoimmunity.[24] Two human anti-CTLA-4 antibodies are currently in early clinical trials (MDX-010, Medarex, and CP-675,206, Pfizer), with evidence that they may have activity in melanoma.[24] There are already a number of antibodies used or being studied as therapeutic agents in cancer as well as autoimmune diseases (eg, alemtuzumab, daclizumab, infliximab, rituximab, epratuzumab).[25–31] Antibodies also can block molecules associated with cell adhesion, thereby inhibiting tumor metastasis.[32,33] With such diverse mechanisms of action, there are a number of opportunities for building antibody-based therapeutics.

Antibodies naturally have long serum half-lives. For immunotherapy, this property is helpful because the antibody is maintained in the body fluids, where it can continually interact with its target. For other targeting strategies, most notably with radioconjugates, it can be harmful because the highly radiosensitive bone marrow is continually exposed to radiation, resulting in dose-limiting myelosuppression. The large size of an antibody impacts its ability to move through a tumor mass. A high interstitial pressure inhibits the diffusion of larger molecules within the tumor.[34] Migration within the tumor is also inhibited by a binding-site barrier, a process where the antibody as it is leaving the tumor's blood vessels binds to the

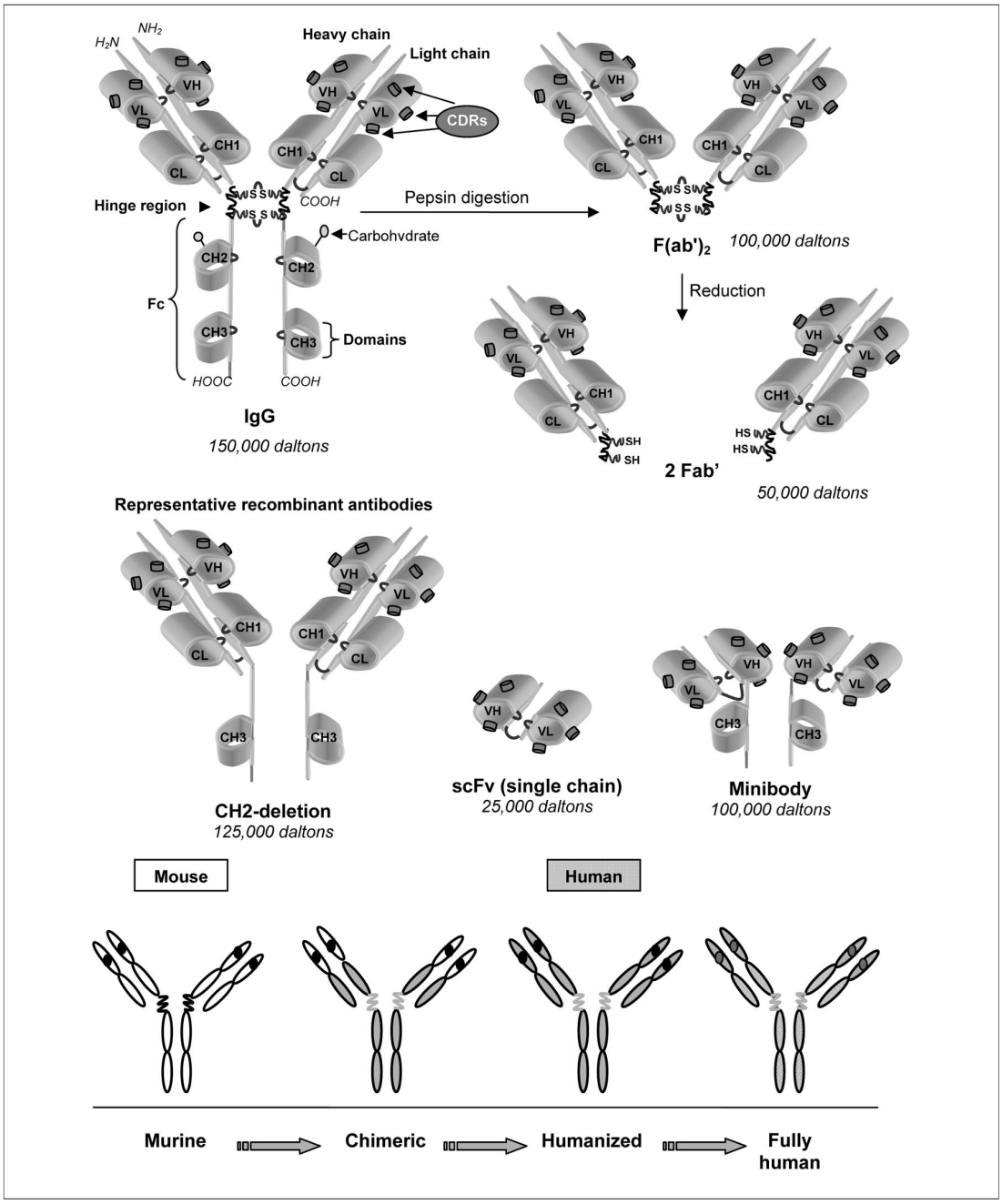

FIGURE 1 Schematic representation of an IgG molecule, its chemically produced fragments, and several recombinant antibody fragments with their nominal molecular weights. At the bottom, a schematic representation of the process involved in engineering murine MAbs to reduce their immunogenicity is provided. A chimeric antibody splices the VL and VH portions of the murine IgG to a human IgG. A humanized antibody splices only the CDR portions from the murine MAb, along with some of the adjacent "framework" regions to help maintain the conformational structure of the CDRs. A fully human IgG can be isolated from specialized transgenic mice bred to produce human IgG after immunizing with tumor antigen or by a specialized phage display method.

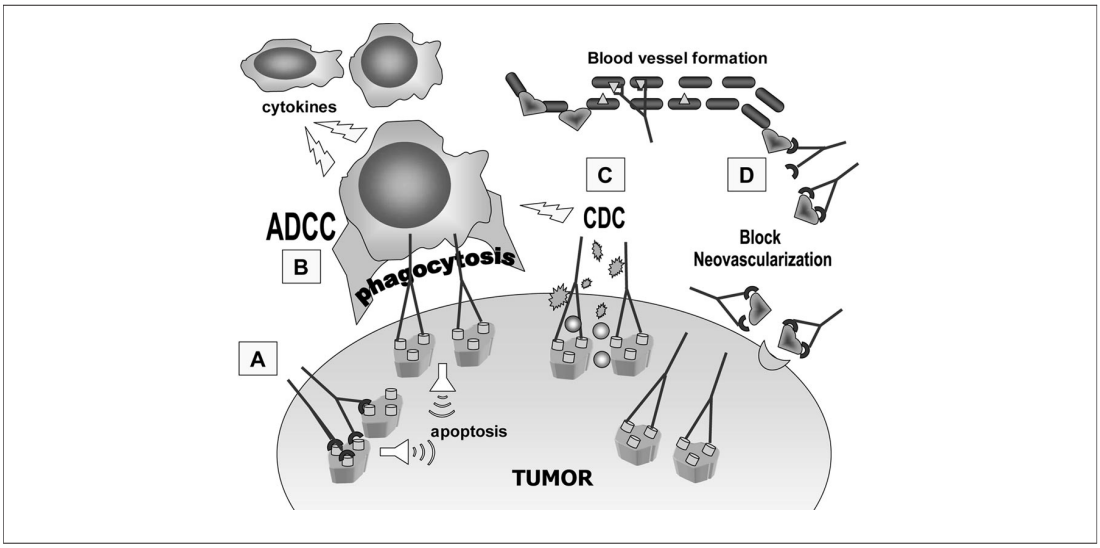

FIGURE **2** Mechanisms of action associated with unconjugated antibodies. In this example, the antigen is shown to be floating in lipid rafts within the tumor cell membrane. (A) Antibodies can activate apoptotic signals by cross-linking antigen, particularly across different lipid rafts. Additional cross-linking of antibody by immune cells can also enhance cellular signaling. (B) Immune cells themselves can attack the antibody-coated cell (eg, phagocytosis), and/or they can liberate additional factors, such as cytokines that attract other cytotoxic cells. (C) If antibodies are positioned closely together, they can initiate the complement cascade that can disrupt the membrane, but some of the complement components also are chemo-attractants for immune effector cells and stimulate blood flow. (D) Tumors also can produce angiogenic factors that initiate neovascularization. Antibodies can neutralize these substances by binding to them, or they can bind directly to unique antigens presented in the new blood vessels, where they could exert similar activities.

first available antigen, concentrating the antibody in the perivascular space.[35] High-affinity antibodies are less likely to migrate into the tumor bed.[36] Administering higher doses of the antibody can reduce the effect of the binding site barrier and allow the antibody to diffuse more deeply into the tumor bed.[37] For cytotoxic agents that must be internalized to kill the cell (eg, toxins, cytotoxic drugs), the ability to distribute throughout the tumor is important. Radioconjugates are less affected by this because some radioactive particles can traverse as much as 1.0 cm from where they are deposited (bystander or crossfire effect).

THERAPY WITH UNCONJUGATED ANTIBODIES

A renewed interest in the effects of unconjugated antibodies in cancer began in the early 1980s, after murine monoclonal antibodies (MAbs) became available.[38] These initial trials were performed in hematological malignancies, as well as in colorectal cancer and melanoma.[4–6,39–41] As with many innovative treatment approaches that are sometimes introduced before the technology has matured sufficiently to extract maximum benefit, only occasional clinical responses were observed. With insufficient efficacy and the immunogenicity of the foreign murine MAb, most of these studies were terminated. Fortunately, some investigators persevered. An excellent lesson on the tribulations of the development of an antibody product between an academic group and industry is that of alemtuzumab (Campath, Berlex, and Genzyme).[42] Alemtuzumab (anti-CD52) had one of the earliest and protracted developments of an antibody ultimately commercialized. It took over 20 years from the development of the first rat immunoglobulin against CD52, changing the immunoglobulin type, and finally developing a humanized, recombinant form, and involved several commercial firms during this time. Chemotherapy-refractive chronic lymphocytic leukemia was the indication finally approved in 2001.

Due in part to the contributions made by the groups led by Morrison (Columbia and Stan-

ford Universities) and Winter (Cambridge), MAbs now are engineered to remove a significant portion of the murine component of the IgG, substituting human IgG components before entering clinical studies.[43–45] Chimeric antibodies essentially splice V_L and V_H regions on the murine antibody to the human IgG, making a molecule that is 75% human and 25% murine IgG, whereas a humanized antibody grafts the CDR regions from a murine MAb, along with some of the surrounding "framework" regions to maintain CDR conformation, onto a human IgG, essentially making a molecule with 5% of its sequence from the parental MAb (Figure 1).[45] More recent advances have made available, either by genetic or phage-display methods, the development of fully human MAbs that have now entered clinical trials.[46] Such engineered MAbs are postulated to greatly reduce the immunogenicity of antibodies, allowing multiple injections to be given, and the human Fc enhances the interaction with other immune system elements.

Rituximab is perhaps the most prominent example of a highly successful paradigm of antibody therapy. As a chimeric antibody, not only did it have reduced immunogenicity, but its effector function (associated with the Fc-portion) was improved. For example, when testing ADCC activity against follicular lymphoma isolated from 43 patients, Weng, et al. reported that only rituximab, not its parent murine anti-CD20 IgG (2B8), had activity in vitro.[47] Rituximab was initially approved as a single agent therapy for relapsed or refractory low-grade, follicular B-cell NHL, having an overall response rate of 48% (10% were complete responses, CR) with a median duration of 11.8 months.[48,49] Since CD20 is not expressed on precursors B-cells, rituximab induces a depletion of only mature B-cells. Rituximab's major side effects, which are thought to be associated with the activation of complement pathways, occur during or shortly after its infusion. Other less common side effects include symptoms associated with tumor lysis syndrome, severe mucotaneous reactions, renal toxicity, cardiac arrhythmias, hypersensitivity reactions, and reactivation of hepatitis B (primarily when used in combination with chemotherapy).[49]

Rituximab's activity is unique among cancer treatments because 40% of the patients retreated with rituximab could again respond with a similar duration.[50] Extending the duration of rituximab therapy can improve the response rate, particularly the number of complete responses, and its duration. However, whether given as a maintenance regimen or retreating at the first sign of progression, the time to chemotherapy was the same.[51] With both approaches having equal benefit, retreatment is generally favored because of the higher expense of a maintenance regimen. Despite the success of rituximab as a monotherapy, there are still a number of patients who do not respond to the initial treatment, and over time, many of those who do will relapse. In an attempt to improve outcome, rituximab has been combined with chemotherapy regimens, including CHOP, CVP, and MCP, as front-line treatments, with very promising results in not only follicular B-cell lymphomas, but also in diffuse large B-cell lymphomas.[52,53] Indeed, trials examining front-line combinations of rituximab and chemotherapy have already demonstrated improvements in response rates, time to progression, and event-free survival, and while the overall response rates are promising based on current 2- to 3-year follow-up data, more time will be required to fully appreciate its impact.[52] Even in chronic lymphocytic leukemia (CLL), where initial testing of rituximab was disappointing, dose intensification and combinations with chemotherapy have provided significant improvements in response.[54,55] Early clinical studies combining rituximab with a humanized anti-CD22, epratuzumab (Immunomedics, Inc.) suggested the potential for additional benefit, particularly in patients with diffuse large B-cell lymphomas.[56,57] Studies have also assessed the possible role of an anti-CD80 MAb (galiximab, biogen idec) as a monotherapy in NHL,[58,59] and clinical trials are in progress testing its combination with rituximab.

Considerable attention has been devoted to understanding the mechanism of action of rituximab, particularly why some B-cell lympho-

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