Minireview

Biological impediments to monoclonal antibody–based cancer immunotherapy

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Abstract
The ability of antibodies to exploit antigenic differences between normal and malignant tissues and to exact a variety of antitumor responses offers significant advantages over conventional forms of therapy. Several monoclonal antibodies (mAb) have already proved to be relatively well tolerated and effective for the treatment of many different malignant diseases. However, mAbs must overcome substantial obstacles to reach antigens presented on target cells to be of therapeutic value. Intravenously administered antibodies must avoid host immune response and contend with low or heterogeneous expression of antigen on tumor cells. Antibodies must also overcome significant physical barriers en route to a solid tumor mass, including the vascular endothelium, stromal barriers, high interstitial pressure, and epithelial barriers. Here we review the application and evolution of mAbs as effective forms of treatment, with particular attention to the barriers and impediments associated with inefficient delivery of mAbs to target cells. We also examine strategies to overcome these barriers and improve the efficacy of mAb–based therapy. [Mol Cancer Ther 2004;3(11):1493–501]

Introduction
Nearly a century ago, Paul Ehrlich first espoused the concept of “magic bullets” for the treatment of disease. By exploiting inherent differences between healthy and diseased cells, therapeutic agents could be designed to specifically target diseased cells while leaving healthy cells unperturbed. While traditional therapeutic modalities, such as chemotoxic drugs or ionizing radiation, are very effective at killing tumor cells, they often fail to adequately discriminate between normal and malignant tissues. Thus, these treatments exact a heavy toll on the patient and can severely limit the dose or duration of treatment to suboptimal levels, resulting in poor outcomes for the management of disease, quality of life, and overall survival. Clearly, elucidating novel therapeutic strategies to target cytotoxicity exclusively to malignant cells would greatly reduce deleterious side effects to normal tissues, enhance the tolerance of the patient to therapy, and result in more effective treatment.

Recent years have seen the emergence of several therapeutic strategies involving monoclonal antibodies (mAb) or related antibody fragments for the treatment of malignancy (1–4). mAbs can be developed with high specificity for antigens expressed on tumor cells and can exact a variety of antitumor responses, thus, the use of mAbs as an alternative or augmentation to therapy offers significant advantages over traditional forms of cancer treatment. While such mAb–based therapies offer a high potential to fulfill the promise of “magic bullets” for the treatment of malignant disease, successful application of these therapies is often hindered by several impediments. Factors inhibiting the therapeutic benefit of mAbs may include low or heterogeneous expression of target antigens by tumor cells, high background expression of antigen on normal cells, host antibody immune responses to the mAbs themselves, insufficient antitumor response after mAb binding, as well as physical obstructions preventing antibody binding, including endothelial, interstitial, and epithelial barriers. This review will focus on the successful application of mAbs as a modality of cancer treatment and will address the barriers and impediments associated with inefficient delivery of mAbs to target cells. We also examine strategies to overcome these obstacles and how the thoughtful and systematic approach to therapeutic design can improve the efficacy of mAb–based treatment of cancer. This review will also provide examples to illustrate key points, and will pay particular attention to prostate specific membrane antigen (PSMA) as a prototypical target for mAb–based therapy.

Tumor-Associated Antigens
By their very nature, antibodies are extremely selective and can exact a cytotoxic effect on target cells. The capacity of the immune system to recognize malignant cells and defend against cancer has long been appreciated. Over 50 years ago, Pressman and Kornfeld (5) showed that antibodies could be used to differentiate between normal and
malignant tissues. Subsequent work by Burnet (6) in the 1960s showed that the immune system could distinguish between normal and malignant cells and that only when lymphocytes lost this capacity would neoplasms actually form. These abilities to target malignant cells and affect an antitumor response form the underlying basis for modern mAb-based cancer therapy.

While tumor cells are ultimately derived from normal progenitor cells, transformation to a malignant phenotype is often accompanied by changes in antigenicity. Expression of tumor-associated antigens (TAA) can arise due to a multiplicity of mechanisms, including alterations in glycosylation patterns (7), expression of virally encoded genes (8), chromosomal translocations (9), or overexpression of cellular oncogenes (10, 11). Hence, the first challenge confronting the development of an effective mAb-based therapeutic strategy is the identification of a suitable TAA with sufficient tumor specificity that is amenable to antibody binding on the surface of target cells.

PSMA is a transmembrane protein expressed primarily on the plasma membrane of prostatic epithelial cells (12). In addition to normal expression within the benign prostatic epithelium, PSMA levels are elevated in virtually all cases of prostatic adenocarcinoma, with highest levels of expression observed in high-grade cancers, metastatic disease, and androgen-independent tumors (12–15). The membrane-associated character and correlation between expression levels and disease grade have enabled PSMA to become an important biomarker for prostate cancer, and antibodies to PSMA are currently being developed for the diagnosis and imaging of recurrent and metastatic prostate cancer as well as for the therapeutic management of malignant disease (15–18).

Members of the epidermal growth factor (EGF) family of receptor tyrosine kinases, in particular HER-2 and epidermal growth factor receptor (EGF-R), also offer attractive targets for therapeutic mAbs. Antibodies to HER-2 are already being used within the clinic for the management of metastatic breast cancer (19, 20). The HER-2 gene is highly overexpressed in approximately 25% to 30% of invasive breast carcinomas (10, 11), and in comparison to normal breast epithelial cells, tumor cells may have up to a 100-fold greater expression of HER-2 (10). Closely related to HER-2, EGF-R is overexpressed in many forms of cancer and, like HER-2, EGF-R is believed to play a significant role in cancer progression (21, 22). Clinical trials are currently ongoing to assess the usefulness of this protein as a target for immunotherapy (21, 23).

B- and T-cell surface antigens are also useful targets for the treatment of malignancy. Many of these antigens, such as CD20, CD22, CD25, CD33, or CD52 are expressed only on a particular lineage of hematopoietic cells (3, 4). These antigens are also expressed at high levels on the surface of various populations of malignant cells, but not on normal tissues or hematopoietic progenitor cells. Therefore, mAbs to these proteins can be used to target both benign mature cells and tumor cells for destruction, while leaving a population of progenitor cells to re-establish normal hematopoietic cell lines (3, 4).

In order for an antigen to be of value for therapy, it must be expressed at sufficiently elevated levels on tumor cells relative to indispensable, normal cells. Inadequate ratios of TAA expression on tumor cells relative to normal cells may severely increase toxicity to the patient and hamper effective treatment. One strategy used to alleviate the problem of low ratios of antigen expression on tumor cells relative to normal cells involves treatment with cytokines, such as interferon α or β. Such treatment has been used to increase expression in tumor cells, while having minimal effect on normal cells (24–26).

**Avoiding Clearance of Therapeutic Antibodies**

Although differences in antigenicity between normal and malignant cells have long been appreciated, the administration of exogenous antibodies for therapeutic benefit did not begin in earnest until the advent of hybridoma technology by Kohler and Milstein (27) in the 1970s. This technology enabled the large-scale preparation of clonal antibodies raised against a specific antigen to be produced in quantities sufficient to be of clinical value (Fig. 1A). Early endeavors using mouse mAbs to combat malignancy met
with some modest degrees of success. Patients receiving doses of mAb experienced very little toxicity, with adverse effects primarily limited to mild allergic reactions. Initial trials also indicated antitumor responses experienced by a fraction of patients, including those refractory to other forms of therapy. Reports of the first full clinical remission in a B-cell lymphoma patient treated with an anti-idiotype mAb further bolstered prospect of using mAbs as an effective treatment for cancer (28).

While many viewed these initial advances as a harbinger of future success, early trials using murine mAbs were plagued by problems resulting in rapid clearance of antibody from the system (29, 30). Typically, i.v. administered murine mAbs exhibited very short half-lives within the serum, generally in the range of 18 to 48 hours (1, 31, 32). This situation was primarily ascribed to the antigenicity associated with the injection of murine antibodies, which tended to elicit the production of a human anti-mouse antibody (HAMA) immune response (30).

Fortunately, advances in molecular biology and genetic engineering have evolved to allay the problems associated with human anti-mouse antibody. Recombinant DNA technology has enabled the creation of less antigenic forms of mAbs, with the majority of the murine immunoglobulin amino acid sequences replaced with those of human proteins. Chimeric antibodies containing only the murine immunoglobulin variable regions fused to human constant domains (33, 34) and humanized mAbs, in which only the actual antigen binding regions of the mouse antibody remain (35), are far less antigenic than mouse mAbs and exhibit longer half-lives within the serum (Fig. 2B and C). The subsequent development of new technologies using phage display libraries (36) or transgenic mice expressing human immunoglobulin genes (37–39) have now enabled the creation of human antibodies (Fig. 2D).

In addition to human anti-mouse antibody, excess antigen within the circulation may also result in rapid clearance of therapeutic mAbs (40). This presence of circulating antigen may be ascribed to a high proportion of TAA expressing normal cells within the vasculature. Alternatively, excess TAA within the circulation may be due to antigen shedding from the cell surface or the presence of secreted isoforms of the TAA. The existence of excess antigen in the serum would sequester i.v. administered mAbs and result in inefficient delivery to tumor cells (40). Ideally, a TAA would be selected that does not undergo antigen shedding from the cell surface or the presence of secreted isoforms of the TAA. In the absence of antigen in the serum would sequester i.v. administered mAbs and result in inefficient delivery to tumor cells (40). Ideally, a TAA would be selected that does not undergo antigen shedding from the cell surface or the presence of secreted isoforms of the TAA.

Treatment of Hematological Malignancies

Following i.v. injection and distribution throughout the vascular space, therapeutic antibodies must access their target antigens on the surface of malignant cells. With few barriers present to impede mAb binding, hematologic malignancies are well suited to mAb-based therapy. In recent years, several promising mAb-based therapies for the treatment of hematologic malignancies have been developed that have received Food and Drug Administration approval or are in advanced phases of clinical testing (1, 3, 4). The chimeric antibody, rituxan (rituximab, Genentech, San Francisco, CA) was among the first mAbs awarded Food and Drug Administration approval for the treatment of non-Hodgkin’s lymphoma (41). This chimeric antibody binds CD20, a cell surface antigen expressed on mature B lymphocytes and over 90% of non-Hodgkin’s lymphoma cells, but not on hematopoietic progenitor or stem cells. Rituxan has proved to be well tolerated and effective in the treatment non-Hodgkin’s lymphoma by itself or in combination with traditional chemotherapy, particularly in patients refractory to other types of therapy (42).

Campath-1 (alemtuzumab, Ilex Oncology, San Antonio, TX) has also received Food and Drug Administration approval for the treatment of patients suffering from chronic lymphocytic leukemia. This humanized mAb recognizes the CD52 antigen present on normal B and T lymphocytes, monocytes, and macrophages, as well as most B- and T-cell lymphomas (43). Campath-1 has shown significant and durable effects in the treatment in patients with previously treated, recurrent, indolent chronic lymphocytic leukemia. A 42% overall response rate was achieved among 29 patients in one phase III study (44). Other encouraging results have demonstrated the efficacy of campath-1 for patients suffering from fludarabine refractory disease (45), and a 73% response rate observed among a limited number of patients with prolymphocytic leukemia (46).

A third mAb to receive Food and Drug Administration approval for the treatment of hematologic malignancies is...
the chimeric mAb, mylotarg (gemtuzumab ozogamicin, Wyeth-Ayerst Laboratories, Philadelphia, PA). This antibody targets the CD33 antigen expressed on myeloid precursors and leukemic blast cells, but like other targets for the treatment of hematologic malignancies, this antigen is absent from normal tissues and pluriotent hematopoetic stem cells (47, 48). Mylotarg has shown promise in the treatment of acute myelogenous leukemia (AML) (49, 50). In addition to rituxan, campath, and mylotarg, several other therapeutic mAbs have been developed and are in various phases of testing, including daclizumab (Zenapax, Hoffmann-LaRoche, Nutley, NJ), a humanized mAb directed against CD25, epratuzumab (LymphoCide, Immunomedics, Morris Plains, NJ), a humanized antibody to CD22.

**Treatment of Solid Tumors**

In comparison to the management of hematologic malignancies, successful treatment of solid tumors with mAbs has proved more elusive; however, some significant therapeutic benefits have been achieved. Herceptin (trastuzumab, Genentech) is a humanized antibody that has received Food and Drug Administration approval for the treatment of metastatic breast cancer. This mAb recognizes an extracellular epitope of the HER-2 protein. Clinical trials with herceptin have shown it to be well tolerated both as a single agent for second or third line therapy, or in combination with chemotherapeutic agents as a first line of therapy. Combination therapy resulted in a 25% improvement of overall survival in patients with HER-2- overexpressing tumors that are refractory to other forms of treatment (19, 20, 51).

The anti-epithelial cellular adhesion molecule mAb, Panorex (eclrecolomab, GlaxoSmith-Kline, United Kingdom), is another mAb-based therapy that is currently being used for the treatment of colorectal cancer. Panorex has shown tangible benefit for cancer patients and has received approval in Germany for the treatment of Dukes’ stage C colorectal cancer (52, 53). Like other mAbs used for the treatment of solid tumors, Panorex has proved more efficacious in the treatment of micrometastatic lesions and minimal residual disease in comparison to bulky tumor masses (52–54).

The failure of mAbs in the treatment of bulky lesions is primarily attributable to the low level of injected mAb that actually reaches its target within a sizable solid tumor mass. Studies using radiolabeled mAbs suggested that only a very small percentage of the original injected antibody dose, approximately 0.01% to 0.1% per gram of tumor tissue, will ever reach target antigens within a solid tumor (55–57). This low level of binding is due to the series of barriers confronted by an i.v. administered mAb en route to TAAs expressed on the surface of tumor cells. These obstacles include the endothelial barrier, interstitial pressure, and stromal impediments, as well as epithelial barriers (Fig. 2). Study into the nature of these impediments has led to increased appreciation for the significance of these barriers and has led to several novel approaches designed to circumvent them.

**Endothelial Barriers**

The microvascular endothelium is lined with endothelial cells, the junctional contacts of which are designed to inhibit passage of macromolecules and cells. To reach antigens expressed on the surface of cells in a solid tumor mass, an i.v. administered mAb must first traverse this formidable barrier. Intratumoral injection is one strategy by which to bypass the endothelium and other physical barriers. Direct intratumoral injection results in increased levels and duration of therapeutic antibodies near the tumor mass with low levels of side effects associated with systemic i.v. injection (58, 59).

Strategies to enhance vascular permeability have been proposed as a method to mitigate the obstructive influence of the microvascular wall and have shown an ability to enhance mAb uptake into solid tumor masses. Pretreatment of patients suffering from extremity localized soft tissue sarcomas and melanomas with the pro-inflammatory cytokine, tumor necrosis factor-α, resulted in an increased perfusion of chemotherapeutic agents into tumors that was associated with improvements in overall tumor response rates (1, 60–63). Unfortunately, systemic administration of tumor necrosis factor-α or other vasoactive agents is severely limited by toxicity, as an adequate dose to increase vascular permeability around a tumor mass is typically 10 to 50 times higher than the maximum dose tolerated by a patient (63, 64). Thus, developing mechanisms to enhance vascular permeability only at sites proximal to a tumor mass would be of great benefit to circumvent this problem.

Intratumoral injection of tumor necrosis factor is capable of increasing the local vascular permeability and improving penetration of therapeutic mAbs in a mouse tumor xenograft model (65). However, such treatment would rarely be a viable clinical option for the treatment of malignancy, because intratumoral injections would be restricted to sites of known, sizable tumor masses. Therefore, mAbs have the potential to fulfill an additional role in cancer treatment to serve as carriers for the delivery of pharmacologic agents to the tumor-associated neovascularure.

Comparable strategies have shown promise to improve the efficacy of mAb therapy. Pretreatment with interleukin-2 (IL-2) conjugated to the TNT-1 mAb directed against the necrotic cells within a solid tumor xenograft resulted in a 3.5-fold enhancement in uptake of a tumor specific radio-labeled mAb without any evidence of toxicity or increase in absorbed dose to normal tissues (55, 66–68). Similar results were obtained using immunonjugates of other vasoactive agents, including recombinant interleukin-1β (IL-1β), physalaemin, histamine, bradykinin, and leukotriene B4 (55). Furthermore, targeted delivery of tumor necrosis factor-α conjugated to the CNGRC peptide of aminopeptidase N (CD13), which targets tumor neovascularure, resulted in an 8- to 10-fold potentiation in the activity of doxycyclin in mouse lymphoma and melanoma models without a significant increase in overall toxicity (64, 69).

In addition to the benign and malignant prostatic epithelium, PSMA is also expressed on the neovascularure associated with a variety of solid tumors, but not in the
normal endothelium (70, 71). By conjugating vasoactive agents to mAbs raised against PSMA, a localized increase in vascular permeability could be achieved exclusively at sites proximal to tumors. Using such a treatment before the administration of therapeutic agents, including antibodies, would substantially enhance the dose received by the tumor.

In addition to enhancing vascular permeability, additional approaches have attempted to exploit intracellular transport pathways as an alternative means by which to bypass the endothelial barrier. McIntosh et al. (72) have used mAbs against antigens found within caveolae of the lung microvasculature to achieve selective and efficient drug delivery to underlying lung tissue.

**Stromal Barriers**

After extravasation across the microvascular wall, a therapeutic mAb must still contend with substantial stromal and interstitial barriers that may result in poor and heterogeneous perfusion throughout a bulky tumor mass. Tumor shape and structure is an important factor influencing mAb uptake, because three-dimensional histocultures, tumor xenografts, and spheroids require longer to reach a steady state concerning protein-bound drug or mAb perfusion, relative to cells in monolayer cultures (73). Tissue composition may also influence mAb uptake, because skeletal muscles serve as restrictive barriers to drug distribution, causing poor perfusion into muscular organs such as the tongue or prostate. The distribution of drugs into these organs is highly heterogeneous, with perfusion limited to areas bordering fibromuscular tissue, and excluded from muscle (73).

An antibody must also contend with the excessive interstitial fluid pressure associated with the high cell density of a sizable tumor mass (74). In studies using spheroids to measure penetration of protein bound drugs, these drugs were primarily restricted to the periphery (73, 75, 76). Thus, high tumor cell density is a major barrier to distribution of macromolecular compounds within solid tumors, and restricts the use of mAb therapy to small volume disease. Reduction of cell density can significantly reduce interstitial fluid pressure, and improve uptake of mAbs and other proteinaceous drugs into the center of tumors (73). Treatment of mice implanted with tumor xenografts with several doses of tumor necrosis factor-α was shown to lower interstitial pressure and improve mAb perfusion by promoting apoptosis of tumor cells (73). Thus, pursuing a treatment regimen that combines several doses of a pro-apoptotic substance with administration of therapeutic mAbs can greatly reduce the interstitial fluid pressure within a tumor and show a synergistic effect compared with a single treatment of pro-apoptotic agents alone.

**Epithelial Barriers**

Epithelial cells are linked together by specialized junctional complexes that provide strong adhesive forces and between neighboring cells and restrict the diffusion of molecules through intercellular spaces. While over 90% of all cancers are carcinomas derived from epithelial tissues, the significance of these barriers is often disregarded in the treatment of malignant disease. However, these physical barriers may exert a profound impact on the efficacy of mAb therapy.

E-cadherin is one of the principal components of the adherens junctions and is of particular relevance to the mAb-based treatment of solid tumors. E-cadherin is a transmembrane protein that mediates adhesion between adjacent cells through a calcium-dependent homophilic interaction (77). These interactions are important for the aggregation of cells and can promote resistance to numerous forms of cancer therapy, including those involving mAbs (78, 79). Adhesion and aggregation among the cells of a solid tumor mass can significantly limit perfusion of therapeutic mAbs and inhibit the penetration of immune effector cells. Disruption of cell-cell adhesion using mAbs to the extracellular domain of E-cadherin can interfere with spheroid formation, and disrupt aggregation of tumor cells expressing E-cadherin (80). Furthermore, use of these anti-E-cadherin mAbs in a murine model was shown to disrupt the structure of tumor xenografts and promote overall survival (81).

Disruption of E-cadherin-mediated cellular adhesion can also be achieved through depletion of extracellular calcium. Administration of the calcium chelator, EDTA, can inhibit interactions between epithelial cells and promote the absorption and penetration of molecules through epithelial tissues (82-84). Furthermore, intratumoral injection of EDTA was showed to enhance the accumulation of the drug cisplatin within tumor xenografts and resulted in a complete cure in four of eight rats, and a substantial reduction in tumor volume for the remaining four rats. In contrast, injection of either EDTA or cisplatin alone had no substantial effect on tumor volume or overall survival (85).

Although targeted disruption of E-cadherin is a potentially valuable strategy to improve the efficacy of mAb-based therapy, it is important to note that E-cadherin is a critical component of normal epithelial tissues and is often down-regulated or absent in many forms of cancer (86, 87). Therefore, it would not be possible to systemically disrupt E-cadherin interaction through i.v. administration of blocking antibodies or other pharmacologic agents. Alternative methods may need to be devised, such that target E-cadherin functions specifically at the sites of tumor cell aggregates. Additional strategies may also include targeting other cellular adhesion molecules, such as N- or P-cadherin, which is typically up-regulated in a variety of cancers (88, 89).

In addition to the adhesive influence of homophilic E-cadherin binding, other complexes, such as the tight junctions, serve to link epithelial cells strongly together. These junctions form regions of close membrane apposition between adjacent cells and physically separate the apical plasma membrane from the basolateral and prevent both the lateral diffusion of proteins and lipids between plasma membrane domains and restrict the flow of proteins, ions, or other solutes through intercellular spaces (46). The basolateral plasma membrane is in contact with adjacent cells and the extracellular basement membrane, and is...
relatively accessible to the solutes within the underlying vasculature. However, because the tight junctions restrict the flow of fluids between cells, i.v. administered agents, such as mAbs, would have very limited access to antigens on the apical plasma membrane. This situation could have a major impact on mAb-based therapy for solid tumors.

Transformation to a malignant phenotype is commonly associated with a down-regulation of E-cadherin expression and a general loss in epithelial polarity and tight junction integrity (86). Following loss of polarity, antigens normally restricted to a particular plasma membrane domain become distributed in a non-polarized fashion throughout the entire surface of the cell. Thus, while administration of mAbs directed against an antigen normally localized to the apical surface would not be readily accessible to normal epithelial cells, these mAbs may be able to preferentially target transformed cells that have lost their polarized phenotype. For instance, the carcinoembryonic antigen (CEA), which is expressed in both normal and malignant cells of the colonic epithelium, is restricted to the apical surface of normal tissues and well-differentiated tumors (90). However, following loss of tight junction integrity in poorly differentiated tumors, CEA is observed in a non-polarized fashion throughout all plasma membrane surfaces (90, 91). The appearance of CEA on the basolateral surface is associated with elevated levels of CEA within the circulation. This elevated level of circulating antigen is presumably due to the increased access of the basolateral surface to the underlying stroma and vasculature (92). This increased expression of CEA on the basolateral surface would also improve accessibility to circulating mAbs, and would explain why immunoscintigraphic studies using i.v. injected mAbs to CEA are able to specifically label primary and metastatic tumors, but not normal or well-differentiated tissues (93, 94).

It is also important to note that while many cells retain polarity as they progress to a malignant phenotype, many tumor cells may retain polarity and have well-differentiated phenotype, even after metastasis to distal sites. Furthermore, tumors are heterogeneous accumulations of cells that are in various stages of differentiation. Thus, while a therapeutic mAb directed against an apical marker may be efficient in targeting undifferentiated cells lacking E-cadherin and tight junctions, populations of well-differentiated tumor or pre-malignant cells may be considerably more resistant to this form of therapy. Such populations of cells may serve as an inoculum, providing a source of cells at various stages of malignancy to repopulate and metastasize to distal sites.

Developing a comprehensive understanding and appreciation for the expression patterns of an antigen within a tumor cell, or the degree of polarization exhibited by a particular population of tumor cells may have a strong prognostic significance and a critical impact on the effectiveness of treatment. A careful screening of tumor biopsies may help to identify those patients who are more likely to respond favorably to a particular mAb-based therapy. For example, the efficacy of treatment of prostate carcinoma with mAbs to PSMA may be highly dependent on the status of the tumor and expression of the antigen. Prostate carcinoma cells often retain high degrees of polarity and tight junction integrity, and PSMA is expressed predominantly on the apical plasma membrane in most normal and malignant tissues (95). However, in situ immunofluorescence revealed that PSMA was also present on the basolateral membrane, with increased accessibility to the vasculature (95). Screening for those patients with tumors that display a non-polarized distribution of PSMA or a general loss of epithelial polarity may identify those who are most likely to respond favorably to such treatment.

**Eliciting a Response**

After successfully negotiating the gauntlet of obstacles obstructing access to the target cells within a tumor, a therapeutic mAb must still be capable of eliciting a potent antitumor response. Although it is often ambiguous as to the exact mechanisms by which a particular mAb may mediate an antitumor response, both direct and indirect mechanisms can potentially be involved.

Antibodies of the IgG1 and IgG3 isotypes can support effector functions of both antibody dependent cell-mediated cytotoxicity and complement dependent cytotoxicity (96). Antibody dependent cell-mediated cytotoxicity is triggered by interaction between the Fc region of a cell-bound antibody and Fcy receptors on immune effector cells such as neutrophils, macrophages, and natural killer cells. This mechanism is critical for the antitumor effects of several therapeutic mAbs, including rituxumab, because mice with normal Fcy receptors exhibit antitumor effects following mAb treatment, while mice lacking Fcy RI and III do not (96, 97).

Many early studies showed that murine mAbs had limited potential to elicit a potent antitumor response, because the murine Fc regions are less efficient at recruiting human effector cells than their human counterparts. This problem has been largely allayed by the use of chimeric and humanized antibodies. Genetic engineering techniques have also been used to improve the immunologic effects of therapeutic mAbs by altering antibody shape and size, increasing the valency of mAbs, and creating bifunctional antibodies with two antigenic receptors, one to a tumor antigen and another to an effector cell to increase efficiency of antibody dependent cell-mediated cytotoxicity (98, 99).

Stimulation of the immune system may also substantially improve effector functions in response to mAb therapy. Sequences of unmethylated bacterial DNA can act as a potent stimulators of immune response (96, 100, 101), and can synergistically enhance the effect of therapeutic mAbs by sensitizing malignant cells to antibody dependent cell-mediated cytotoxicity (102). Furthermore, different oligonucleotide sequences were shown to illicit different immune responses, suggesting that mAbs are capable of inducing antitumor effects by a variety of mechanisms.

In addition to immunologic effects, mAbs can induce antitumor effects by a variety of direct mechanisms, including the induction of apoptosis (103, 104), or the prevention of soluble growth factors from binding their...
cognate receptors, such as EGF-R (23) and HER-2 (105, 106). Additionally, mAbs can also be engineered to deliver a cytotoxic agent directly to the tumor. This offers the potential to combine the biological effects of mAbs with the additional effect of a targeted cytotoxic response. The anti-CD33 mAb, mylotarg, is one such antibody. Combined with the cytotoxic agent, calicheamicin, mylotarg has been reported to be relatively well tolerated, and effective in the treatment of chronic lymphocytic leukemia (107). Antibodies can also be engineered to deliver ionizing radiation directly to tumor cells. mAbs to the extracellular domain of PSMA have been conjugated to both α- and β-particle emitting radionuclides, and have shown promise in the treatment of xenograft-bearing mice (16–18, 108). Clinical trials in humans also portend the promise of radiolabeled mAbs for the treatment of cancer. In a recent phase III randomized study, patients with relapsed or refractory low-grade non-Hodgkin’s lymphoma were treated with a yttrium-90 and an iodine-131-labeled mAb to CD20 (ibrituzumomab tiuxetan and tositumomab, respectively). Patients treated with these radiolabeled mAbs showed a statistically significant increase in overall response compared with those treated with an unlabeled version of the mAb (rituximab) (109).

Conclusion
The remarkable specificity and ability to affect an anti-tumor response indicate that mAbs offer great promise to fulfill the role of “magic bullets” in the treatment of malignancy. These agents have already become an important clinical modality in the treatment of many forms of hematologic malignancies, and some solid tumors, as well. The successes to date are a likely a mere prelude to future application of mAbs to cancer therapy. Unfortunately, the successful application of mAbs to therapy is largely inhibited by a series of formidable obstacles that prevent antibodies from reaching their target antigens on the cell surface. Hopefully, a greater appreciation of these barriers will be developed and systemic approaches to overcome these barriers will lead to improved efficacy.

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References
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