Review

Immunotherapy of Malignant Disease with Tumor Antigen–Specific Monoclonal Antibodies

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Abstract

A few tumor antigen (TA)–specific monoclonal antibodies (mAb) have been approved by the Food and Drug Administration for the treatment of several major malignant diseases and are commercially available. Once in the clinic, mAbs have an average success rate of ~30% and are well tolerated. These results have changed the face of cancer therapy, bringing us closer to more specific and more effective biological therapy of cancer. The challenge facing tumor immunologists at present is represented by the identification of the mechanism(s) underlying the patients' differential clinical response to mAb-based immunotherapy. This information is expected to lead to the development of criteria to select patients to be treated with mAb-based immunotherapy. In the past, in vitro and in vivo evidence has shown that TA-specific mAbs can mediate their therapeutic effect by inducing tumor cell apoptosis, inhibiting the targeted antigen function, blocking tumor cell signaling, and/or mediating complement- or cell-dependent lysis of tumor cells. More recent evidence suggests that TA-specific mAb can induce TA-specific cytotoxic T-cell responses by enhancing TA uptake by dendritic cells and cross-priming of T cells. In this review, we briefly summarize the TA-specific mAbs that have received Food and Drug Administration approval. Next, we review the potential mechanisms underlying the therapeutic efficacy of TA-specific mAbs with emphasis on the induction of TA-specific cellular immune responses and their potential to contribute to the clinical efficacy of TA-specific mAb-based immunotherapy. Lastly, we discuss the potential negative effect of immune escape mechanisms on the clinical efficacy of TA-specific mAb-based immunotherapy. Clin Cancer Res; 16(1); 11–20. ©2010 AACR.

The concept that antibodies could be used for the treatment of malignant disease originated more than a century ago (1). The first generation of antibody-based therapies were based on the use of tumor antigen (TA)–specific allogeneic, autologous, or xenogeneic polyclonal antibodies, which were ill suited as cancer-specific therapies because of their limited or lack of specificity and reproducibility. It was not until the development of the hybridoma technology (2) that antibody-based immunotherapy of malignant diseases became a practical reality. The hybridoma technology enabled the production of a large number of human TA–specific murine monoclonal antibodies (mAb). The clinical application of some of them yielded a handful of promising results that were however overshadowed by disappointing outcomes in early clinical trials implemented with TA-specific mouse mAb in patients with various types of cancer (3). In hindsight, the inadequate response rates (RR) observed with first generation TA-specific mAb most likely reflected their murine origins, resulting in high immunogenicity and poor ability to recruit immune effector mechanisms (4–6). These hurdles have been recently overcome by the generation of chimeric, humanized, and human mAb, resulting in reduced or lack of immunogenicity and improved ability to recruit effector cells (7).

Today, TA-specific mAb have been established as highly sensitive and reproducible probes in the diagnostic arena (8–13) as well as in clinically and commercially successful therapies for a variety of malignant diseases (3). The average clinical success rate of TA-specific mAb-based immunotherapy, which manifests itself as statistically significant disease-free interval and survival prolongation as well as reduction of tumor mass in some of the treated patients, is ~30% with ranges from 0% to 60% (3, 14). Only little is known about why merely a limited percentage of the treated patients respond clinically to TA-specific mAb-based immunotherapy. The mechanisms underlying the patients' differential clinical response to TA-specific mAb-based immunotherapy represent a topic of intense research at present, because this information has both basic research and clinical relevance. It will contribute to our understanding of the molecular basis of the clinical efficacy of TA-specific mAb-based immunotherapy and it will lead to the development of criteria to select patients to be treated with TA-specific mAb-based immunotherapy. In this review, we first summarize the...
Translational Relevance

Tumor antigen–specific monoclonal antibodies (mAb) have been successfully implemented into standard treatment regimens for patients with a variety of malignant diseases. Despite the clinical successes, scant information is available about the variables underlying patients’ differential clinical response to mAb-based immunotherapy. The scant information in this area has a negative effect on the optimization of the use of tumor antigen–specific mAb in therapeutic strategies and represents a major obstacle to the selection of patients to be treated with mAb-based immunotherapy. This review discusses the potential mechanisms underlying the therapeutic efficacy of mAb-based immunotherapy in patients with malignant disease. This information is expected to contribute to our understanding of the molecular basis of the clinical efficacy of mAb-based immunotherapy and to lead to the development of criteria to select patients with malignant disease to be treated with mAb-based immunotherapy.

TA-Specific mAb

In recent years, the regulatory approval and sales of new human medicines indicates an increasing number of biological therapies, i.e., small molecular inhibitors and mAb specifically designed to target malignant cells (15). In fact, the global sale of mAb–derived biological therapies in 2006 was $20.6 billion dollars, indicating a major paradigm shift in industrial research and development from pharmaceutical to biological therapies (15). At the end of 2007, >30 years of clinical studies have resulted in the approval of six unconjugated, humanized, or chimeric TA-specific mAbs for cancer therapy along with one drug immunonconjugate and two radioisotope immunoconjugates (Table 1; refs. 3, 14). The results of clinical trials in patients treated with TA-specific mAb have shown that the goal of cancer-targeted therapy is realistic and may be superior to older nonspecific conventional chemotherapy- and radiotherapy-based regimens, at a minimum enhancing their activity. Once in the clinic, TA-specific mAbs are well tolerated with an average success rate of 30% (3, 14). Clinical responses have been found to include complete and partial responses as well as statistically significant increases in progression-free survival and in disease-free interval and survival in patients with several malignant diseases (Table 1). In general, compared with solid tumors, hematologic neoplasms have been proven easier to target with TA-specific mAb-based therapies because therapeutic efficacy can be achieved at lower doses and tumor penetration is more readily achieved (3, 14). Moreover, radioimmunotherapy has been more successful for hematologic malignancies such as non–Hodgkin’s lymphoma. However, fewer of these agents are entering clinical trials due to complexities in manufacture and safety concerns compared with unconjugated mAb (3, 14). An additional limitation of radioimmunotherapy relates to the delivery of the dose of radioactivity to the tumor compared with normal tissues (3, 14). The poor specificity index is due to slow pharmacokinetics and slow tumor perfusion.

Compared with traditional chemotherapy- and radiotherapy-based regimens, in general, the side effects of immunotherapy with nonconjugated TA-specific mAb are fairly mild (3, 14). Nonetheless, toxicities do occur and may be classified as mechanism-independent and mechanism-dependent (Table 2). Most of the toxicities related to TA-specific mAb are mechanism-independent and are related to allergic or hypersensitivity reactions caused by a protein containing xenogeneic sequences (3, 14). These reactions occur during or just after the first injection and are summarized in Table 2. Moreover, human anti-mouse immunoglobulin antibodies, including anti-idiotypic antibodies, can complex with circulating therapeutic mAb, and inhibit their targeting of tumor cells. It should be noted that conjugated antibodies often have a lower therapeutic index than nonconjugated TA-specific mAb, and for this reason, they often result in more side effects (3, 14). Rare, but more serious side effects of TA-specific mAb-based immunotherapy are often related to mechanism-dependent toxicities and result from the binding of a therapeutic antibody to its target antigen (Table 2; refs. 3, 14).

Molecular Mechanisms Underlying the Therapeutic Efficacy of TA-Specific mAb-Based Immunotherapy

The majority of nonconjugated TA-specific mAb approved for clinical use display intrinsic antitumor effects mediated by one or more of the mechanisms outlined in Table 3. They can be broadly divided into those that require immune effector cells and those that do not. It should be noted that these mechanisms do not function independently, but extensively interact with each other. The relative importance of each mechanism varies with the type of tumor and the treatment administered. Moreover, it should be stressed that TA-specific mAb have been clearly shown to be able to inhibit their specific receptor and induce apoptosis in the targeted tumor cell without
the influence of immune cells in vitro. Nevertheless, it is still debated whether immune effector cells or receptor blockade is the dominant mode of action in vivo or if both pathways need to cooperate to achieve therapeutic effect.

TA-specific mAb can block activation signals that are needed for continued malignant cell growth and/or viability by blocking the interactions between the ligand and its receptor, inducing modulation of the receptor or interfering with ligand binding and/or dimerization of the receptor (reviewed in refs. 3, 14). The latter mechanisms seem to be particularly important for the epidermal growth factor receptor (EGFR)– (16–21), CD20– (22–26), and vascular endothelial growth factor–specific (27–30) mAb. Alternatively, some TA-specific mAb may exert their effects through Fc-based mechanisms such as antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC; refs. 31, 32). ADCC and CDC are dependent on interactions of antibody Fc domains with cellular Fcγ receptors (FcγR) expressed on immune accessory cells and with the classic complement-activating protein C1q, respectively. The potential to mediate ADCC and CDC is a function of an antibody’s subclass and is also influenced by the nature of its glycosylation (33). Triggering of both ADCC and CDC not only activates natural killer (NK) cells, neutrophils, mononuclear phagocytes, and/or dendritic cells (DC), but also induces secretion of IFN-γ, tumor necrosis factor-α, chemokines, and opsonins that recruit immune effector cells. Consequently, tumor cell proliferation and angiogenesis are inhibited; antigen presentation is increased; and tumor cells are lysed (31, 32, 34).

Several of the clinically approved TA-specific mAbs, such as rituximab, cetuximab, trastuzumab, alemtuzumab, panitumumab, and 131I-tositumomab, have been shown to activate ADCC and CDC in vitro and when administered to mice transplanted with TA-expressing tumor cells. In many cases, it is difficult to unravel whether the therapeutic efficacy of TA-specific mAb depends more on ADCC or CDC; however, there has been some work in this area. It is noteworthy that the most convincing evidence for ADCC and CDC in the antitumor activity of TA-specific mAb comes from hematologic malignancies, i.e., the CD52- and CD20-specific mAbs alemtuzumab and rituximab, respectively (3, 14). Whether this finding reflects the accessibility of tumor cells to both TA-specific mAb and plasma proteins of the complement cascade as well as immune effector cells remains to be determined. Nevertheless the role of CDC in the antitumor activity of mAb, which recognize antigens expressed by malignant lymphoid cells, is suggested by experimental and clinical findings. First, alemtuzumab mediates significant CDC of chronic lymphocytic leukemia (CLL) cells in vitro (35). Second, the ability of rituximab to cure immunocompetent mice challenged with murine lymphoma EL4 cells stably transfected

| Table 1. FDA-approved TA-specific mAb for human cancers |
|----------------|----------------|----------------|----------------|----------------|
| mAb            | Target         | Isotype        | FDA-approved disease |
| Rituximab      | CD20           | Chimeric IgG1  | CD20 (+) low-grade lymphoma,* diffuse large B-cell lymphoma,* follicular lymphoma* |
| 90Y Ibritumomab + tiuxetan | CD20           | Radiolabeled murine IgG1 | CD20(+)* low-grade lymphoma† |
| 131I Tositumomab | CD20           | Radiolabeled murine IgG1 | CD20(+)* low-grade lymphoma† |
| Alemtuzumab    | CD53           | Humanized IgG1 | Chronic lymphocytic leukemia‡ |
| Gemtuzumab + ozogamicin | CD33       | Recombinant humanized IgG4-conjugated to calicheamicin | Acute myelogenous leukemia§ |
| Trastuzumab    | HER2/neu       | Humanized IgG1 | Her2/neu (+) breast cancer¹ |
| Cetuximab      | EGFR           | Humanized IgG1 | EGFR(+) Colon cancer¹ |
| Panitumumab    | EGFR           | Fully human IgG2 | EGFR(+) Colon cancer¹ |
| Bevacizumab    | VEGF           | Humanized IgG1 | Colon cancer,** recurrent or advance non–small cell lung cancer, metastatic breast cancer |

Abbreviations: FDA, Food and Drug Administration; VEGF, vascular endothelial growth factor.

*Low-grade lymphoma: second-line monotherapy; diffuse large B-cell lymphoma and follicular lymphoma: first-line chemoimmunotherapy therapy as well as maintenance for follicular lymphoma.

¹Second-line monotherapy.

²First- and second-line monotherapy.

³>60 y of age, second-line monotherapy.

⁴Second-line monotherapy, adjuvant, and first-line chemoimmunotherapy.

⁵Second-line monotherapy or chemoimmunotherapy.

⁶First-line chemoimmunotherapy.

**First-line chemoimmunotherapy.
with human CD20 is completely abolished in syngeneic knockout mice lacking C1q (36). Lastly, complement consumption has been observed after administration of the CD20-specific mAb rituximab to patients with lymphoma (32, 37). It is noteworthy that target antigen density seems to be a critical factor for CDC, since Golay et al. (39) have shown that the success of rituximab in mediating CDC against malignant B cells is highly dependent on CD20 density. Whether the lack of convincing evidence for the role of CDC in the antitumor activity of TA-specific mAb used for solid tumors reflects inadequate TA density remains to be determined.

The role of ADCC in the antitumor activity of TA-specific mAb is also suggested by experimental and clinical findings. First, transgenic mice that lack type I and type III FcγR have provided the conclusive evidence that mAbs are capable of targeting immune effector cells to cancer cells in vivo (31, 39). In this regard, FcγR (−) mice, unlike wild-type mice, fail to show protective immunity against tumor challenge using several antigen/antibody systems. Furthermore, the removal of the Fc portion from TA-specific mAb reduces TA-specific mAb-related side effects as well as their antitumor activity. In contrast, deletion of the inhibitory type II FcγR (FcγRIIb) results in an increased protective effect, suggesting that FcγRIIb modulates TA-specific ADCC activity in vivo (31). The role of interactions with cellular FcγR in the clinical efficacy of TA-specific mAb-based immunotherapy is further supported by the statistically significant correlation between improved clinical RR to mAb-based immunotherapy and particular “high responder” FcγR polymorphisms in patients with (a) CD20(+) follicular cell lymphoma treated with rituximab, (b) metastatic colon cancer treated with cetuximab, or (c) metastatic breast cancer treated with trastuzumab (3). Nevertheless, it should be stressed that the type of FcγR polymorphism does not seem to be associated with improved clinical RR in every patient with a certain disease and in every disease. For example, Fcγ

### Table 2. Toxicities related to TA-specific mAb for human cancers

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<tr>
<th>Mechanism dependent</th>
<th>Rituximab</th>
<th>90Y Ibritumomab + tiuxetan</th>
<th>131I Tositumomab</th>
<th>Alemtuzumab</th>
<th>Gemtuzumab + ozogamicin</th>
<th>Trastuzumab</th>
<th>Cetuximab</th>
<th>Panitumumab</th>
<th>Bevacizumab</th>
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<tr>
<td>Mechanism dependent</td>
<td>Transitory lymphocyte B depletion</td>
<td>Reactivation of hepatitis B/C, cytomegalovirus, and parvovirus B19</td>
<td>Transitory lymphocyte B depletion</td>
<td>Pancytopenia</td>
<td>Myelosuppression</td>
<td>Cardiac dysfunction</td>
<td>Severe infusion reactions</td>
<td>Severe infusion reactions</td>
<td>Hypertension, proteinuria, minor bleeding, or thrombosis</td>
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<td>Mechanism independent</td>
<td>Reversible ventricular tachycardia</td>
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<td>Autoimmune idiopathic thrombocytopenia and hemolytic anemia</td>
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<td>Flushing, seborrheic dermatitis, and acniform eruptions</td>
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<td>Pulmonary fibrosis</td>
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*Caused by mAb-containing xenogeneic sequences. These reactions occur during or just after the first injection.
†Patients must have >25% bone marrow involvement to be considered eligible for treatment.
‡Pneumocystis jiroveci pneumonia and herpes virus prophylaxis is recommended for patients being treated with alemtuzumab.
§When administered in combination with paclitaxel or an anthracycline and cyclophosphamide.
∥Rare and usually occur within minutes of the initial infusion.
¶Alone or in combination with chemotherapy.
**In patients treated within 60 d from surgery.
RIIIa-158F polymorphism is an indicator of improved clinical RR in patients with colon cancer and breast cancer treated with cetuximab and trastuzumab, respectively, but is linked to poor RR in patients with hematologic malignancies treated with rituximab (3). Moreover, FcγRII and RIII polymorphisms are not associated with improved clinical RR in chronic lymphocytic leukemia patients treated with alemtuzumab or rituximab, in diffuse large B-cell lymphoma patients treated with rituximab, and in follicular cell lymphoma non–Hodgkin’s lymphoma patients treated with sequential cyclophosphamide-Vincristine-Adriamycin-Prednisone (Oncovin) and rituximab (3). Whether these conflicting findings reflect the effect of other variables, such as characteristics of the TA-specific mAb used, induction of TA-specific CTLs, tumor sensitivity to apoptosis, and host immune cell dysfunction, on the role of FcγR polymorphisms in the patients’ clinical response to antibody-based immunotherapy is not known.

At present, the variables underlying the differential clinical RR of the patients treated with antibody-based immunotherapy have not been identified. The data we have summarized suggest that ADCC and CDC play a part in the clinical efficacy of at least some TA-specific mAbs. However, other mechanism(s) are likely to underlie the durable clinical responses observed in some patients treated with TA-specific mAbs, because every tumor cell is not completely eradicated during therapy with TA-specific mAbs. Given the number of interactions that are predicted to occur among different components of the immune system, several investigators have begun to examine the ability of TA-specific mAb to induce TA-specific cytotoxic T-cell responses in TA-specific mAb-treated patients. In this regard, TA-specific mAbs are likely to enhance antigen-presenting cell (APC) internalization and antigen presentation of TA, as well as cross-priming of T cells through endocytosis and phagocytosis of TA-containing immune complexes and antibody-opsonized tumor target cells, respectively (40, 41). Furthermore, the activation of both ADCC as well as CDC can further augment and focus the generation of TA-specific T-cell immunity through the production of cytokines, chemokines, and opsonins, which ultimately lead to (a) amplification of ADCC and CDC, (b) recruitment and activation of immune effector cells, (c) maturation of APC, (d) enhancement of antigen presentation, and (d) generation of TA-specific CD4(+) and CD8(+) T-cell immunity (Fig. 1). Moreover, generation of cleavage products from components of the complement pathway, e.g., C3b, may activate complement receptor 3 on the surface of effector cells and induce complement receptor 3–dependent cellular cytotoxicity (42).

The possibility that TA-specific mAb can enhance the immunogenicity of TA and induce TA-specific cellular immunity is supported by the following lines of preclinical and clinical evidence. First, incubation of ovarian cancer cells with the CA125-specific mouse IgG1 mAb oregovomab in vitro can induce CA125-specific and autologous ovarian TA-specific CTL (43). Second, cross-presentation mediated by FcγRs on human DC can enhance the presentation of multiple myeloma antigens to patient-derived T cells, thus suggesting that uptake of antibody-opsonized tumor cells and cellular fragments by APCs could lead to antigen/epitope spreading and induction of immunity to several TA (40). Third, in vitro incubation of cells with trastuzumab results in augmentation of HER2-specific CTL killing of HER2(+) tumors, through the ability of trastuzumab to mediate HER2 internalization and to enhance HLA class I antigen–restricted presentation of endogenous HER2 through proteasomal processing (44). Fourth, immunization of mice with DC pulsed with antibody-opsonized TA acquired through either FcγR-mediated endocytosis (40, 42, 43) or phagocytosis (43) induces CD4(+) and CD8(+) T-cell–mediated tumor immunity. Lastly, induction of a TA-specific CTL response has been recently documented in patients treated with TA-specific mAb-based immunotherapy (46). Six of 10 evaluable breast cancer patients showed augmented HER2-specific CD4 T-cell responses during therapy with trastuzumab. Furthermore, the number of patients with detectable HER2-specific antibodies among the 27 treated with trastuzumab

Table 3. Molecular mechanisms underlying therapeutic efficacy of TA-specific mAb-based therapy

<table>
<thead>
<tr>
<th>Immune effector cell independent*</th>
<th>Immune effector cell dependent</th>
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<tr>
<td>Induce apoptosis</td>
<td>Activation of complement mediated:</td>
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<td>Induce alteration in intracellular signaling</td>
<td>Phagocytosis</td>
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<td>Inhibit growth factor binding to its cognate receptor</td>
<td>CDC</td>
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<td>Inhibit growth factor receptor activation</td>
<td>Trigger antibody-dependent cellular cytotoxicity</td>
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<td>Induction of tumor cell necrosis or apoptosis leading to</td>
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<td>Presentation of TA by APC</td>
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<td>Activation of CD(+) T-cell–mediated kill</td>
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<td>Activation of B cells and eosinophils</td>
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<td>Activation of TA-specific CTL</td>
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*Ultimately results in inhibition of cancer cell proliferation, tumor-induced angiogenesis, cancer cell invasion and metastasis, as well as potentiating antitumor activity of cytotoxic drugs and radiotherapy.
increased from 8 (29%) before treatment to 15 (56%) during treatment ($P < 0.001$). Similar results have been obtained by one of us (RLF) in studies in progress. Specifically, HLA class I antigen–restricted, TA-specific CTL were detected in four of seven HLA-A*0201+ patients with head and neck squamous cell cancer treated with the EGFR-specific mAb cetuximab.

**Fig. 1.** Triggering of immune effector mechanisms by TA-specific mAb-based immunotherapy. TA-specific mAb may participate in host-dependent immune effector mechanisms including (A) activation of (i) complement-mediated phagocytosis and/or (ii) CDC, (B) induction of ADCC, and/or (C) induction of tumor cell necrosis or apoptosis. The latter mechanisms result in the release of TA as well as the production of cytokines and opsonins that lead to (i) TA uptake, APC maturation and presentation of TA by APC, (ii) generation of CD8(+) TA-specific CTL through CD4(+) T-cell help, and (iii) activation of CD4(+) T helper cells which leads to activation of NK cells, granulocytes, and macrophages through release of Th1 cytokines, CD4(+) T-cell–mediated killing, and activation of B cells and eosinophils through release of Th2 cytokines.

FcyR, have been reported to present HLA class I antigen–restricted exogenous antigens (47, 48). An alternative, although not exclusive, possibility is represented by the induction of anti-idiotypic antibodies, i.e., antibodies specific for the variable region of the administered TA-specific mAb (49, 50). In some cases, these anti-idiotypic antibodies may mimic the TA recognized by the original TA-specific mAb and function as a surrogate antigen (49, 50). The anti-idiotypic antibody as such or complexed with the TA-specific mAb may be taken up by professional APC and induce a TA-specific T-cell immune response (49, 50).

**Tumor Cell Resistance to TA-Specific CTL**

Despite appropriate TA expression, patients may not have a clinical response to TA-specific mAb-based immunotherapy and/or may develop resistance to therapy during
the course of treatment. The multiple mechanisms by which TA-specific mAb may exert their antitumor effects make it difficult to determine which "escape" mechanism(s) is/are most important in patients. Furthermore, the intrinsic genetic instability of tumor cells (51) limits our ability to predict how successful TA-specific mAb-based immunotherapy will be. Nevertheless, several mechanisms of tumor cell resistance to TA-specific mAb-based immunotherapy have been suggested (reviewed in refs. 3, 14). For the purpose of this review, we have focused on the ability of tumor cells to evade immune recognition and destruction by TA-specific CTL.

If T cells do play a role in the clinical efficacy of TA-specific mAb-based immunotherapy, then the escape mechanisms, which have been identified in patients treated with T-cell–based immunotherapies, will have relevance. The latter include changes in TA, HLA antigen, and antigen-processing machinery component expression as well as CD4(+)CD25(+) T regulatory cells, each of which modulates the interaction between tumor cells and T cells. Specifically, because of their genetic instability (51), tumor cells may change in the expression of molecules such as TA, HLA class I antigens, and/or antigen-processing machinery components; all of them play a crucial role in the generation of the HLA class I antigen–TA peptide complex, which mediates the recognition of tumor cells by the host’s CTL. Approximately 10% to 30% of tumor cells may fail to express TA and a variable degree of interlesional and intralesional heterogeneity may also be present in a patient (52). Furthermore, tumor cells may present TA-derived peptide analogues with antagonist activity resulting in suboptimal T-cell activation (53). These defects have been found to render malignant cells ineffective targets for TA-specific T cells. Moreover, HLA class I antigen downregulation or loss by tumor cells occurs with a frequency of about 10% to 80% depending on the type of malignancy (53). These abnormalities are caused by distinct molecular mechanisms (reviewed in ref. 54). In some of these cases, HLA class I antigen expression can be restored by cytokines, providing the potential for benefit by combining TA-specific mAb-based immunotherapy with administration of cytokines.

**Fig. 2.** Induction of TA-specific CTL responses by TA-specific mAb-based immunotherapy. TA-specific mAb may enhance the generation and promote the survival of TA-specific CTL through several mechanisms. TA-specific mAb may (i) induce tumor cell death or activate (ii) ADCC and (iii) CDC. The latter results in (a) the formation of the lytic membrane-attack complex (MAC); (b) the generation of opsonins (C3b), and (d) the release of the anaphylatoxins C3a and C5a. The culmination of the above events (i-iii) leads to the release of Th1 cytokines, (iv) the formation of TA-specific mAb-TA complexes, and (v) the uptake of TA and TA-specific mAb-TA complexes by APC. Ultimately, mature DC present processed TA to CD4(+) and CD8(+) T cells, and promote the generation of (vi) TA-specific CTL.
It is noteworthy that the role of HLA class I antigen abnormalities in the clinical course of the disease is highlighted by their increased frequency in recurrent cancers in patients treated with T-cell–based immunotherapy (53). These findings have important implications if the generation of TA-specific CTL underlies, at least in part, the clinical efficacy of TA-specific mAb-based immunotherapy. Specifically, the loss of HLA class I antigens will facilitate the outgrowth of tumor cells that have acquired the ability to escape T-cell recognition because of these defects. If this interpretation is correct, the outgrowth of tumor cells with HLA class I antigen abnormalities is likely to reflect escape of tumor cells from immune recognition more than dormancy or ignorance of the patient's immune system. This possibility is supported by the disease progression frequently observed in patients with malignancy in spite of the development and/or persistence of a TA-specific CTL response (54). Moreover, given the high rate of mutations in tumor cells, the generation of TA-specific CTL through TA-specific mAb therapy, even when successful, is not likely to be able to completely eradicate a patient's malignancy, because the eventual outcome is likely to be outgrowth of tumor cells with HLA class I antigen abnormalities.

HLA class I antigen–TA peptide complex loss by tumor cells may not solely be responsible for their immune resistance because in several cases, TA-specific CTL coexist with cancer cells expressing the target components required for recognition (55). Factors such as the type of T-cell response induced (i.e., tolerance), lack of CD4+ helper-T-cell activation, host immune cell dysfunction, activating antigen-specific CD4(+)CD25(+) T regulatory cells, production of inflammatory and lytic molecules by tumor cells, and/or high tumor burden may also be responsible for the lack of association between self–TA-specific T-cell responses and clinical outcome (55–59).

Toward Improving Immunotherapy

Clearly we are at an early stage in our understanding of the molecular mechanisms by which TA-specific mAb-based immunotherapy mediates effective clinical responses. Although not conclusive, the information we have reviewed has focused attention on the potential role of TA-specific adaptive immunity in patients treated with TA-specific mAb-based immunotherapy. Such responses would be desirable, because they provide a mechanism for long-term protection and immunologic memory. Moreover, the generation of TA-specific adaptive immunity provides a mechanism to explain the improved clinical responses in at least some patients treated with repeated administrations of some TA-specific mAb.

If T cells do play a role in the clinical efficacy of TA-specific mAb-based immunotherapy, one might ask why T cells activated in this setting are effective at controlling tumor cell growth but not when patients are vaccinated with T-cell–based activation strategies. Several possibilities can be envisioned to underlie the ability of TA-specific mAb to induce more clinically effective TA-specific T-cell immune responses. First, the type of TA targeted by the TA-specific T-cell responses generated by TA-specific mAb-based immunotherapy may be very important. In this regard, most of the currently used TA in T-cell–based immunotherapy trials represent differentiation and/or shared TA that have been identified from tumor metastases and CTL from patients who, in the majority of cases, have failed to reject their cancer. Moreover, many of these TA are selected on the basis of their immunogenicity and tissue distribution without paying much attention to their function in tumor cell biology. Whether any of the identified TA are tumor rejection antigens and whether stage IV cancer patients represent the most appropriate source to identify clinically relevant TA is not known at present. Second, TA-specific mAbs have the potential to generate adaptive immune responses against the entire TA repertoire of a particular patient's tumor because they are able to enhance cross-presentation of multiple TA through their direct anti-tumor effects. Third, the adaptive immunity potentially generated by TA-specific mAb provides a means to match therapeutic regimens with changing tumor antigenic profiles that are likely to occur during the course of the disease. Fourth, the potential ability of TA-specific mAb to generate adaptive immunity against multiple TA increases the chances to also target unique TAs that have been suggested to be more effective targets of T-cell–based immunotherapy than shared TA (60). Lastly, the polyvalent vaccine–like potential of TA-specific mAb not only eliminates the requirement for patient selection based on HLA type, but may also be able to counteract escape mechanisms caused by selective loss of HLA class I allospecificities and TA. However, it should be noted that one disadvantage of the use of TA-specific mAb is the lack of knowledge about the identity of the TA-mediating tumor recognition that are targeted in adaptive immune responses; the lack of this information hinders the standardization of the assays designed to monitor immune responses in patients treated with TA-specific mAb-based immunotherapy.

The ability of TA-specific mAb to induce more clinically effective TA-specific T-cell immune responses may also stem from their ability to activate multiple arms of the host's immune system including ADCC, CDC, innate immune effector cells, DC, NK, B cells, and T cells. This is in contrast to the narrow view of strictly activating TA-specific CTL that has been attempted in the majority of the T-cell–based vaccine strategies to date. In this regard, recent progress into our understanding of the mechanisms underlying activation and proliferation of the adaptive arm of the immune response provides support for the concept that tumor cells may directly and/or indirectly lead to dysfunction and/or death of immune cells in the tumor microenvironment (55). Therefore, it is unlikely that T-cell–based vaccination strategies will be successful in the setting of tumor cell–induced immune suppression. Through their ability to activate ADCC, CDC, and APC, TA-specific mAbs may promote a Th1 cytokine–rich environment, thereby enhancing both the
number and function of DC, B cells, as well as NK cells and ultimately preventing premature death of T-cell effectors. It is noteworthy that the potential ability of TA-specific mAb to activate NK cells may prove significant, because there is growing evidence that NK cells are required for the activation of DC as well as the generation of antigen-specific T- and B-cell response both in vitro and in vivo (61–63).

If it is determined that the induction of such TA-specific T-cell immune responses does contribute to the clinical efficacy of TA-specific mAb-based immunotherapy, in vivo induction of ADCC or CDC leading to TA-specific CTL could be viewed as potential biomarkers of clinical response. In this regard, it should be noted that the ability of TA-specific mAb to induce ADCC, CDC, and TA-specific T-cell immune responses might have important implications for the development of future TA-specific mAb. As noted above, the potential of a TA-specific mAb to mediate ADCC and CDC is a function of not only its subclass but also its degree and type of glycosylation (33). Notably, deglycosylated antibodies are unable to bind FcγR and the presence of sialic acid residues confers anti-inflammatory properties to mAb. Therefore, the type and amount of glycosylation of TA-specific mAb is likely to play an important role in balancing the proinflammatory versus anti-inflammatory properties of administered antibodies. It is possible that this vaccine-like property of TA-specific mAb-based immunotherapy could be exploited to selectively amplify or bias the resulting adaptive immune response by promoting antigen presentation, host antibody production, and expansion of TA-specific CTL. Nonetheless, it must be kept in mind that if indeed T cells are major players in the clinical efficacy of TA-specific mAb-based immunotherapy, we will continue to face the negative effect on the outcome of immunotherapy of escape mechanisms selected for by immune pressure. Consequently, even when successful, it is likely that immunotherapy will facilitate the emergence and expansion of tumor cell populations with TA and/or HLA antigen defects and eventually the recurrence of malignant lesions. The latter suggests that immunotherapy for the treatment of cancer may only be successful in a limited number of patients. Even when successful, it is likely that the selective pressure imposed by immunotherapy will facilitate the emergence and expansion of tumor cell populations with TA and/or HLA antigen defects and, eventually, the recurrence of malignant lesions. Therefore, it will be important to combine immunotherapy with other types of immunologic and nonimmunologic strategies, which use distinct mechanisms to control tumor growth. In this regard, the concomitant targeting of other cells in the tumor microenvironment, which are crucial for malignant cell survival and proliferation, may counteract the negative effect of tumor cell genetic instability on immunologic and nonimmunologic therapies.

Disclosure of Potential Conflicts of Interest

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