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REVIEW ARTICLE

Tumor Antigen–Targeted, Monoclonal Antibody–Based Immunotherapy: Clinical Response, Cellular Immunity, and Immunoescape

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ABSTRACT

Purpose
Tumor antigen (TA) –targeted monoclonal antibodies (mAb), rituximab, trastuzumab, and cetuximab, are clinically effective for some advanced malignancies, especially in conjunction with chemotherapy and/or radiotherapy. However, these results are only seen in a subset (20% to 30%) of patients. We discuss the immunologic mechanism(s) underlying these clinical findings and their potential role in the variability in patients' clinical response.

Methods
We reviewed the evidence indicating that the effects of TA-targeted mAb-based immunotherapy are mediated not only by inhibition of signaling pathways, but also by cell-mediated cytotoxicity triggered by the infused TA-targeted mAb. We analyzed the immunologic variables that can influence the outcome of antibody-dependent cell-mediated cytotoxicity (ADCC) in vitro and in animal model systems. We also analyzed the correlation reported between these variables and the clinical response to mAb-based immunotherapy.

Results
Of the variables that influence ADCC mediated by TA-targeted mAb, only polymorphisms of Fcγ receptors (FcγR) expressed by patients' lymphocytes were correlated with clinical efficacy. However, this correlation is not absolute and is not observed in all malignancies. Thus other variables may be responsible for the antitumor effects seen in mAb-treated patients. We discuss the evidence that triggering of TA-specific cellular immunity by TA-targeted mAb, in conjunction with immune escape mechanisms used by tumor cells, may contribute to the differential clinical responses to mAb-based immunotherapy.

Conclusion
Identification of the mechanism(s) underlying the clinical response of patients with cancer treated with TA-targeted mAb is crucial to optimizing their application in the clinic and to selecting the patients most likely to benefit from their use.

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INTRODUCTION

Convincing evidence indicates that tumor antigen (TA) –targeted monoclonal antibody (mAb) –based immunotherapy, using rituximab (anti-CD20), trastuzumab (anti–human epidermal growth factor 2 [HER2]), and cetuximab (anti–human epidermal growth factor 1 [HER1]/epidermal growth factor receptor [EGFR]), is clinically effective in lymphoma, breast cancer, and head and neck (HNC) and colorectal carcinomas (CRC), respectively.[1-9] Despite the disparate etiologies leading to the development of these malignancies, mAb therapy provides clinical response rates and a survival advantage in each of them,[10] and their therapeutic efficacy is often enhanced by combination with radiotherapy or chemotherapy[11-14] These findings have restored confidence among clinical oncologists in the value of biologic therapy for the treatment of malignant disease and have facilitated enrollment in clinical trials with TA-targeted mAb. As a result, during the last few years, a large number of patients have been treated with TA-targeted mAb-based immunotherapy. Two findings are noteworthy. First, although the antigens used as targets are expressed by a large number of normal cells, administration of TA-targeted mAb causes adverse effects, including allergic reactions to the introduced foreign proteins, only in a limited number of patients. Second, as single agents, TA-targeted mAbs yield response rates of 8% to 10% in advanced, heavily pretreated and recurrent disease[10]; their therapeutic

Table 1. Selected FDA-Approved TA-Targeted mAbs for Human Cancers

mAb	Target	Isotype	FDA-Approved Diseases
Cetuximab	EGFR/HER1	Chimeric IgG1	EGFR-positive colon cancer, HNC
Panitumumab	EGFR/HER1	Fully human IgG2	EGFR-positive colon cancer, HNC
Rituximab	CD20	Chimeric IgG1	$CD20^+$ low-grade lymphoma, diffuse large B-cell lymphoma, follicular lymphoma
Trastuzumab	HER2/*neu*	Humanized IgG1	HER2/*neu*-positive breast cancer

Abbreviations: FDA, US Food and Drug Administration; mAbs, monoclonal antibodies; EGFR, epidermal growth factor receptor; HER, human epidermal growth factor receptor; Ig, immunoglobulin; HNC, head and neck cancer.

efficacy is often enhanced by combination with radiotherapy or chemotherapy, with the response rate increasing up to 30%. A related observation is that efficacy is seen in only some of the malignant diseases expressing the targeted TA on tumor cells.

These findings raise the question of which mechanism(s) underlie(s) the therapeutic efficacy of TA-targeted mAb-based immunotherapy. Answers to this question have both theoretical and practical implications. On one hand, it will contribute to our understanding of why TA-targeted mAb-based immunotherapy has a differential clinical effect on patients with a given type of malignant disease and why it works in only some of the diseases that express the targeted TA on tumor cells. On the other hand, it will likely define criteria to select patients to be treated with TA-targeted mAb-based immunotherapy, to monitor their clinical response, and to optimize the immunotherapy schedule.

We first review the evidence indicating that not only inhibition of signal transduction pathways, but also immunologic mechanisms, underlie the antitumor activity of currently used TA-targeted mAbs. Then we describe the variables that influence the extent of cell-dependent lysis of target cells mediated by TA-targeted mAb in vitro and in animal models. Finally, we discuss the clinical relevance of these variables as well as the experimental[15] and clinical evidence[16] that argues for a role of TA-targeted cellular immunity triggered by TA-targeted mAb, and immunoescape mechanisms, in the clinical outcome of TA-targeted mAb-based immunotherapy.

CLINICAL ACTIVITY OF TA-TARGETED mAbs

A large number of reviews describe the clinical efficacy of the therapeutic, TA-targeted mAbs, rituximab, trastuzumab, and cetuximab, in lymphoid and epithelial malignancies (Table 1).[7,17-21] Although TA-targeted mAbs may be used as single agents, most clinical scenarios use these mAbs in conjunction with radiotherapy and/or chemotherapy and demonstrate enhancement of clinical activity as compared with conventional therapy when given without the mAb.[4,7,22,23] The clinical efficacy of mAb-based immunotherapy is manifested by higher cure rates in previously untreated patients with cancer and prolongation of overall survival in patients with recurrent/metastatic disease.[8,9,24] Clinical response is observed in mAb-treated patients over $\geq$ 1 weeks, a time frame consistent with a T-lymphocyte–mediated lytic effect, and these kinetics coincide with the transport of TA to the draining lymph node in vivo within 48 hours.[25-27] In mAb-treated patients with cancer, tumor lysis syndrome is rarely observed over the course of hours or days,[28] as would be expected for purely natural killer cell (NK cell) –mediated lytic effects. In addition, blockade of Erb-B receptor activation (phosphorylation) occurs within 10 to 20 minutes of mAb treatment in vitro,[29,30] arguing for additional mechanisms in the clinical responses observed in these patients in vivo. The reduction in relative risk of death or recurrence is between 20% and 30% with the addition of cetuximab, trastuzumab, or rituximab to radiotherapy[4] or chemotherapy.[5,31,32] Recently, administration of trastuzumab has been combined with HER2 peptide vaccines to augment the clinical activity of the mAb,[12] and adjuvant therapies used in breast carcinoma do not seem to reduce the activity of TA-specific T cells.[12,33] Recurrence of disease in patients demonstrating initial clinical response is likely to represent escape of tumor cells from the antitumor effect exerted by the TA-targeted mAb used.[1-6,34] Because the clinical efficacy of these TA-targeted mAbs has been well-described elsewhere,[7,17-21] we will focus on the potential role that immunologic and immune escape mechanisms play in the differential clinical response to mAb-based immunotherapy.

MECHANISMS UNDERLYING THE ANTITUMOR ACTIVITY OF TA-TARGETED mAbs

TA Expression and Signaling Blockade Mediated by TA-Targeted mAbs

CD20, EGFR (ErbB1, HER1), and HER2 (ErbB2) are the molecules targeted by rituximab, cetuximab, and trastuzumab, respectively, which are clinically effective in the treatment of lymphoma, HNC and CRC, and breast cancer, respectively. These targets share several features. First, they are expressed on both normal and malignant cells, although the latter express much higher levels of TA, with mutations occurring only in rare cases.[22] These quantitative differences appear to play a role in the pathogenesis of the disease, because an association has been found between increased levels of these antigens and prognosis. An additional similarity among these clinically efficacious mAbs as therapeutic agents seems to be their targeting of surface receptors involved in downstream signal transduction. EGFR and HER2 are tyrosine kinase receptors that belong to the Erb-B/HER receptor family. HER1 and HER2 initiate signaling through several pathways, including the phosphatidylinositol 3-kinase(PI3K)/AKT and Ras/mitogen-activated protein (MAP) kinase pathway, which promote cell survival and proliferation.[35] CD20, the targeted antigen of rituximab, has been suggested to trigger antiapoptotic pathways in B cells through Bcl-2.[34,36] In addition, in CRC, cetuximab clinical efficacy is significantly predicted by the absence of activating k-RAS mutations, in addition to some predictive power of Fcγ receptor (FcγR) polymorphisms for clinical response in these patients.[8,37] This mechanism(s) is (are) not likely to play a major role in HNC, which also benefits from the therapeutic activity of cetuximab, because this disease does not manifest appreciable mutations in k-RAS (or EGFR[38,39]). These findings support the use of receptor tyrosine kinase inhibitors (TKI) for the treatment of EGFR-overexpressing diseases. However, lack of target specificity of TKIs for ErbB family kinases, and their generally lower clinical activity than mAbs recognizing these

targets, have focused attention on mAb-based therapies directed at this family of growth factor receptors. In addition, the kinetics of clinical response observed in mAb-treated patients results in tumor shrinkage over weeks, consistent with a T-lymphocyte–mediated lytic effect, not usually over the course of hours, as would be expected for purely NK cell–mediated effects. Furthermore, the nearly complete reduction in Erb-B receptor activation (phosphorylation) within 10 to 20 minutes of mAb blockade of ligand binding in vitro[38,40,41] also argues for additional mechanisms besides signaling inhibition in the clinical responses observed in these patients in vivo.

IMMUNOLOGIC VARIABLES MODULATING THE EXTENT OF ANTITUMOR ACTIVITY OF TA-TARGETED mAbs

Several lines of evidence suggest that blockade of signal transduction may not be the only mechanism of action mediating clinical benefit of mAb-treated patients with cancer.[35,42,43] Indeed, the potential role of immunologic mechanisms in the therapeutic efficacy of ErbB-targeted mAb (as opposed to TKI) is supported by several lines of evidence. First, tumor cell apoptosis is not observed in vitro without the addition of lymphocytes to the culture system.[40] Second, correlation of clinical response is observed in patients expressing certain polymorphisms of mAb-binding receptors on NK cells, monocytes, and granulocytes known to have lytic activity. Last, biomarkers of clinical response, such as level of expression, activation, or genomic amplification of EGFR, have not been consistently correlated with clinical response to mAb therapy targeting these receptors,[44] indicating that other mechanisms may explain the observed clinical activity.

Among the variables known to play a role in the antitumor activity of TA-targeted mAbs is their ability to mediate lysis of tumor cells in vitro by NK cells, monocytes, and granulocytes in an antibody-dependent cell-mediated cytotoxicity (ADCC) assay. The extent of lysis in this assay is in turn influenced by several variables, and they, or at least some of them, may contribute to the differential clinical response of patients treated with mAb-based immunotherapy. Many of these variables have been characterized preclinically in vitro and in animal model systems. In particular, the role of B cells and complement has been investigated, but is not the primary focus of this review.[45-47] The available information will be reviewed regarding patients' clinical responses to TA-targeted mAb-based immunotherapy.

Expression and Density of the Targeted TA on Tumor Cell Surface

In a number of tumor types, the expression level of the targeted TA (CD20, HER2, or EGFR) on target cells has been shown to influence the extent of their mAb-mediated lysis in vitro, especially when the effector cells (ie, NK cells and monocytes) display low lytic efficiency.[40,48] However, in most studies, allogeneic tumor cells with different levels of the targeted TA have been used as targets.[49] Therefore, the potential interference of confounding variables cannot be excluded. At any rate, the available data are at variance with the conflicting results about the relationship between the expression level of the targeted TA (CD20, HER2, or EGFR) on tumors and clinical response. Although clinical responses to EGFR-targeted mAb, cetuximab, are generally not correlated with level of EGFR expression on tumor cells,[43] Burtness et al[50] showed an inverse correlation with EGFR expression, and Chung et al[51] even showed clinical activity in EGFR-negative tumors. One might argue that the discrepancy between the results of the in vitro studies and the clinical findings reflects the lack of sensitivity of the method used, generally standard immunohistochemical staining, to measure target antigen expression in malignant lesions. However, an alternative possibility we favor is the role of additional immunologic variables,[41,52-54] as described below.

Influence of mAb Isotype and Dose on the Induction of TA-Targeted Cellular Immunity and Antitumor Activity

In addition to FcγR polymorphism, mAb concentration, association constant, and, most importantly, isotype subclass play an important role in the extent of cell-dependent lysis of target cells, with human immunoglobulin (Ig) G1 and IgG3 being more efficient at mediating lysis of target cells than IgG2 and IgG4 isotypes (Table 2).[48,55] A dose-response relationship has been identified for mAb antitumor activity in vitro.[48,49] Although the dose relationship plateaus above 10 μg/mL, plasma levels are 50 to 150 μg/mL, indicating sufficient circulating mAb available for maximal tumor cell binding in patients. When a single TA is targeted by mAbs of different IgG isotype, variability in immune activation may be mediated by differential FcγR binding affinity. In this regard, a unique example is the two mAbs targeting EGFR currently approved by the US Food and Drug Administration, cetuximab (IgG1 isotype) and panitumumab (IgG2 isotype). These two mAbs compete for the same ligand binding site(s) on the ecto-domain of EGFR, but the IgG2 isotype of the latter is predicted to result in lower ability to induce cellular immune reactions (Table 2).[40,56] A recent preclinical study did demonstrate, unexpectedly, the ability of panitumumab to mediate ADCC through myeloid-derived granulocytes, including neutrophils.[55] Further clinical studies using panitumumab in CRC and HNC are underway to clarify whether this represents an actual mechanism of antitumor activity in patients.

Table 2. mAb Isotype Binding Affinity to Polymorphic FcγR Subtypes

		CD32			CD16	
Characteristic	CD64, FcγRI	FcγRIIa	FcγRIIb	FcγRIIc	FcγRIIIa	FcγRIV
Affinity	High	Medium	Low	Low	Medium	Medium
Specificity	High	Low	Medium	Low	Medium	High
Isotype that binds preferably	IgG1, IgG3	IgG1, IgG3	IgG1, IgG2	IgG2a, IgG2b	IgG1	IgG2a, IgG2b
Comments		131-R/R genotype has lower affinity for IgG1	Inhibitory receptor		176-F/F most common genotype, associated with lower affinity for IgG1	

Abbreviations: mAb, monoclonal antibody; FcγR, Fcγ receptor; Ig, immunoglobulin.

Table 3. Molecular Mechanisms Underlying Therapeutic Efficacy of TA-Targeted mAbs

Triggering of antibody-dependent cellular cytotoxicity
Activation of:
Phagocytosis
Complement-dependent cytotoxicity
DC maturation and TA uptake
Induction of cellular immunity leading to:
Presentation of TA by antigen-presenting cell (ie, DC)
Activation of $CD4^+$ T-cell–mediated killing
Activation of B cells and eosinophils
Activation of TA-targeted cytotoxic T lymphocytes

Abbreviations: TA, tumor antigen; mAbs, monoclonal antibodies; DC, dendritic cell.

Complement-dependent lysis (CDC) may also be observed in vitro using IgG1 isotype TA-targeted mAb[57] and has been proposed to mediate effects of rituximab, trastuzumab, and more recently cetuximab in vitro and in murine systems, particularly in association with B cells.[45-47] However, the rapid effects of CDC question its major role in clinical responses to mAb-based immunotherapy, which are usually observed over ≥ 1 weeks. CDC has also been suggested to play a major role in some of the adverse effects observed.[57-64] However, this mechanism of action does not provide an explanation for variability in patient responses to the mAb used (Table 3).

FcγR Polymorphisms and Disease Status

Interactions between tumor cells coated with TA-targeted mAbs and effector cells such as NK cells are mediated by FcγR.[65] These receptors are expressed by monocytes and NK cells, the major effector cells in mAb-mediated lysis of tumor cells.[1-3,37,66] The functional significance of FcγR polymorphisms is highlighted by its association with the extent of in vitro lysis of target cells in ADCC and with the control of growth of human tumors grafted in immunodeficient mice.[2,3, 43,67] Among the activating receptors, FcγR IIIa is expressed on NK cells and FcγR IIa on monocyte-derived dendritic cells (DC), B cells, and granulocytic cells. Little clinical information is available regarding the role of inhibitory (IIb) FcγR in mAb-treated patients with cancer; this topic is an intriguing area of potential investigation. Clinical activity of TA-targeted mAb seems to be associated with patients who harbor particular so-called high-responder FcγR IIa/IIIa H- and V- encoding polymorphisms, arguing for a role of FcγR in the clinical efficacy of at least some of these malignancies.[11] The molecular basis for the differential binding of the Fc portion of Abs to polymorphic FcγR reflects the substitution of histidine (H) with arginine (R) in codon 131 of FcγRIIa and of valine (V) with phenylalanine (F) in codon 158 of FcγR IIIa.[68,69] In a murine xenograft model of breast cancer[70] and HNC (R.L. Ferris, unpublished data), the antitumor effects of trastuzumab and cetuximab, respectively, depend in part on the presence of FcγR-bearing immune cells, including NK cells.[2,3] Only limited information is available about the effect of disease status on function of effector cells, but in vitro comparison of the lytic activity of peripheral-blood mononuclear cells from healthy donors and from patients with cancer suggests that the latter have reduced ability to lyse mAb-coated tumor cells. These defects can be corrected in vitro with cytokines such as interleukin (IL) -2, IL-15, and IL-21, which are known to enhance NK cell expression of FcγR.[48,49,71]

It should be stressed that polymorphic genotypes of FcγR do not seem to be associated with improved clinical outcome in every patient and every disease. For example, the FcγRIIa-131 H polymorphism seems to be indicative of improved response rate in patients with breast carcinoma, CRC, and follicular lymphoma treated with trastuzumab, cetuximab, and rituximab, respectively. In contrast, the FcγRIIIa-158F polymorphism is correlated with improved response rates in patients with CRC[72] treated with cetuximab, but is also linked to poor response rates in patients with breast carcinoma treated with trastuzumab,[1] CRC treated with cetuximab,[37] and hematologic malignancies treated with rituximab.[3,73] Moreover, FcγRIIa/IIIa polymorphisms are not associated with clinical outcome in patients with chronic lymphocytic leukemia treated with rituximab or alemtuzumab, in patients with diffuse large B-cell lymphoma treated with rituximab, and in patients with follicular lymphoma treated with sequential cyclophosphamide, doxorubicin, vincristine, and prednisone chemotherapy and rituximab. Moreover, relevance of this immunologic mechanism of action to clinical efficacy is correlative, and NK cell-mediated lysis occurs in vitro over 4 to 6 hours, a time frame that would be consistent with a more rapid tumor lysis and clinical response than that observed in patients with cancer (a week or more). Lastly, even when clinical responses are observed, this is not seen in every patient whose lymphocytes express favorable FcγR genotype and whose tumor expresses the targeted TA.[3,37,73] In addition, as mentioned, conflicting data have been published in CRC[37,72] for the allele with best predictive ability for cetuximab clinical response. These results argue in favor of a role for other variables, in addition to the FcγR polymorphism, in the clinical response to mAb-based immunotherapy.

Potential Role of TA-Targeted Cellular Immunity in the Clinical Efficacy of mAb-Based Immunotherapy

Most of the variables found to influence ADCC of tumor cells by FcγR-bearing effector cells in vitro and in animal model systems are not associated with clinical responses.[74,75] In addition, the association of patients' FcγR genotype with clinical outcome to mAb therapy is significant, but not absolute. These clinical findings support the role of additional mechanisms in the clinical responses observed in patients with cancer treated with these agents. As mentioned above, generation of TA-mediated cellular immunity in vivo is consistent with the kinetics of clinical responses observed in treated patients with cancer. An increasing number of results in animal model systems and in clinical settings indicates that TA-targeted antibodies trigger or enhance TA-targeted cellular immune responses,[15,16] involving cytotoxic T lymphocytes (CTL) and helper (Th) T cells.[16,74,76-79] This evidence is reviewed below.

FcγR and Antigen Presentation

Therapeutic mAbs are effective in enhancing antigen cross-presentation by DC to T cells in vitro and in vivo, resulting in augmentation of TA-targeted CTL generation. A growing body of evidence suggests that the uptake, internalization, and presentation of apoptotic cell-derived or soluble TA to $CD8^+$ T cells by DC are enhanced by various receptor(s) on DC that have endocytic activity[80] and by activating FcγR such as FcγRI, IIa, and III.[16,74,78,81] Antigen uptake, in the form of immune complexes or opsonized tumor cells, is associated with enhanced antigen presentation by DC,[74,78] as well as by B cells,

which express FcγR. Depending on the associated costimulatory signals, these effects can be stimulatory or suppressive of TA-specific activation, leading to cellular immunity or plasma cell induction (humoral immunity). Thus treatment of patient with cancer with mAbs may trigger a TA-targeted $CD8^+$ T-cell response by enhancing the antigen uptake through FcγR on DC in the microenvironment or draining nodes. The detection of TA-targeted CTL in mice after immunotherapy with mAb[78,81] and of a $CD4^+$ T-cell response in patients with breast cancer treated with trastuzumab[16] support the hypothesis that TA-targeted mAb induce TA-specific T-cell responses in vivo. It has also been suggested that the FcγRIIa polymorphism plays a role in mAb-mediated TA cross-presentation by DC.[27,74,76,77]

NK Cell–DC Crosstalk Enhancement of Cellular Immunity Triggered by TA-Targeted mAbs

Potential mechanisms of enhanced cross-presentation induced by mAb-based immunotherapy include facilitation of TA:mAb complex uptake by DC, enhancement of FcγR ligation and stimulation of DC,[74,76] induction of costimulatory and adhesion molecules on the DC surface, and upregulation of antigen processing machinery (APM) components known to be crucial for optimal TA processing and presentation.[82,83]

Although most innate immune cells express both inhibitory and activating FcγR, NK cells constitutively express only a low-affinity, activating FcγRIIIa (CD16), which initiates lytic activity on encountering mAb-coated targets. In addition to mediating ADCC, the subpopulation of NK cells[84] characterized by high CD56 expression, referred to as $CD56^{bright}$, secretes T helper type 1 (Th1) cytokines, such as interferon γ, tumor necrosis factor α, and chemokines, such as macrophage inflammatory protein-(MIP)-1α, MIP-1β, and RANTES, that inhibit tumor cell proliferation, enhance antigen presentation, and aid in the chemotaxis of T cells.[48,85-87] NK cell–DC cross-talk follows the recruitment of both NK cells and DC to sites of inflammation,[87,88] potentially reducing the activity and number of immunosuppressive, regulatory T cells (known as suppressor T cells; Tregs).[52,89] The resulting potent activating bidirectional signaling can shape both the innate immune response within inflamed peripheral tissues and the adaptive immune response in secondary lymphoid organs. Additionally, NK cells in the presence of cytokines released by DC become activated, regulating both the quality and the intensity of innate immune responses. In turn, DC in the presence of cytokines released by activated NK cells enhance cross-presentation and priming of T cells. In conclusion, through direct interactions and secretion of cytokines/chemokines,[48,86,87] NK cells may function as helper cells[90] and enhance and broaden T-cell priming against multiple TA,[91] including the targeted TA as well as "private" TA. The latter type of TA has been suggested by results obtained in animal model systems to be more efficient than shared TA in mediating tumor rejection by T-cell immunity. Their clinical application, however, is hampered by the limited progress made in their identification and by the practical difficulties to implement immunotherapeutic therapies individualized for each patient.

Enhancement of TA Cross-Presentation by DC to T Cells in the Presence of TA-Targeted mAbs

Although many investigators have explored the induction of ADCC, a growing body of evidence, as well as our preliminary results, indicate that TA-targeted mAb, including cetuximab, rituximab, and trastuzumab, can effectively trigger TA-specific CTL responses. In preclinical studies,[25] mAbs recognizing a TA have been shown to activate targeted TA-specific $CD8^+$ T-cell responses. This activation is thought to occur via the processing of exogenously acquired antigens by antigen-presenting cells followed by presentation on major histocompatibility complex (MHC) class I antigens to $CD8^+$ T cells, termed cross-presentation. DCs in particular efficiently process externally acquired antigens and present them via enhanced cross-priming on MHC class I molecules on their cell surface to enhance $CD8^+$ T-cell activation (the effector cells that ultimately recognize and lyse antigen-expressing tumors). It has been reported that a mAb recognizing the rat HER2/*neu* antigen expressed by murine mammary tumor cells can induce TA uptake and cross-priming that correlated with improved in vivo tumor rejection.[25]

Interestingly, in mice the $CD8^-$ population of DC does not typically cross-prime unless specifically activated via its Fcγ receptor,[27] a finding consistent with the in vitro data presented above. DC express the low-affinity FcγR III, the high-affinity FcγR I, and the complement receptor 3 and mannose receptor, and these receptor-mediated phagocytic mechanisms require formation of immune complexes. These immune complexes bind directly to FcγR to initiate phagocytic signal transduction,[92] activation of FcγR through the binding of immune complexes, and enhanced antigen presentation in DC.[16,76-78,93,94] By enhancing the uptake of TA by DC and their presentation to T cells,[74,77,78,81] these emerging new data suggest that the generation of HLA class I restricted, TA-targeted T cells triggered by therapeutic mAbs may be influenced by the FcγR expressed by NK cells and monocyte-derived DCs.[76-78] In addition, mAb:TA complexes may enhance the induction of DC cross-presentation by inducing or upregulating the expression of APM components and costimulatory molecules associated with maturation phenotype of DC.[82,95,96] Enhancement of DC maturation programs and upregulation of APM components known to be highly correlated with optimal TA cross-presentation,[82] such as TAP1/2, are strongly induced by incubation with cetuximab and activated NK cells.[76]

POTENTIAL ROLE OF IMMUNE ESCAPE IN THE LACK OF CLINICAL RESPONSE TO mAb-BASED IMMUNOTHERAPY

Clinical responses to TA-targeted mAb-based immunotherapy are correlated with the patients' particular FcγR genotypes[72]; however, tumor progression often occurs in these patients. Escape mechanisms used by tumor cells to evade mAb-induced antitumor immunity may play a role in patients' differential clinical response to TA-targeted mAb-based immunotherapy.[97] For instance, mAb-mediated tumor cell lysis may be influenced by tumor cell expression of NK cell inhibitory proteins, such as HLA-E[98] and HLA-G.[99,100] It has been shown that NK cell dysfunction is frequently observed in patients with cancer and especially in those with advanced disease.[101,102] This variable could influence the extent of lysis in ADCC independently of the FcγRIIIa polymorphism. Inhibitory signals transmitted to NK cells and CTL may provide a mechanism of immune escape by tumor cells, such as through Treg cells, as shown in multiple cancer types.[99,100,103,104] In addition, rituximab-mediated NK cell lysis has been recently shown to be inhibited by HLA-G mediated interference with NK cell activation and tumor cell killing.[105] Thus analysis of classical and nonclassical HLA class I[106] antigen expression by tumor

cells, which can influence NK cell lysis,[107,108] should contribute to define the mechanisms underlying differential responses to mAb-mediated antitumor immune effects in vitro and in vivo.

We note that nonimmune escape from TA-targeted mAb therapy may occur, mediated by accessory signaling pathways that compensate for blockade of the pathways downstream of the targeted TA. Indeed, recent evidence from cetuximab-resistant tumor systems indicates that treatment escape may be mediated by upregulation of G-protein coupled receptors that can bypass the inhibited Erb-B receptor targeted by the mAb[30,109-111] or expression of other HER family receptors, such as HER2 or HER3.[112,113] This is corroborated by the value of elevated levels of ErbB family ligands as predictors of clinical response to trastuzumab and cetuximab.[10,114]

Impact of APM Component Defects on In Vivo Tumor Cell Recognition by HLA Class I–Restricted, TA-Targeted CTL

APM plays a crucial role in the generation of HLA class I-TA–derived peptide complexes expressed by antigen-presenting cells. In tumor cells or in DCs cross-presenting TA, the APM generates peptides from mostly, although not exclusively, endogenous TA, which are presented by surface MHC molecules to cognate CTL. Abnormalities in the expression and/or function of APM components have been found in malignant cells with a frequency of 50% to 70%[115,116]; these defects result in scrambled or altered expression of trimolecular complexes on the surface of tumor cells, thereby allowing for tumor cell escape from T-cell recognition. The recently[16] described induction of TA-specific T cells in mAb-treated patients with cancer provides the rationale for suggesting that APM defects in tumor lesions play a role in the differential clinical response to cetuximab-based immunotherapy.[77,78,107,116-118]

ROLE OF CELLULAR NETWORKS IN mAb-MEDIATED ANTITUMOR ACTIVITIES

In summary, mAb immunotherapy is clinically effective, but there is significant variability among patients' responses. Tumor signaling pathways do not adequately explain the variability in clinical response observed nor exclude other potential mechanisms of antitumor activity. Immunologic mechanisms, such as ADCC, are modulated by mAb-binding, polymorphic FcγR on immune cells, level of TA expression by tumor cells, concentration of mAb used, and frequency and reactivity of immune cells in the tumor microenvironment, including TA-targeted CTL and Tregs. In addition, various potential mechanisms of escape from TA-targeted mAb therapy have been detected to enable avoidance of TA recognition or immune cell-dependent tumor lysis. A model we propose for cellular cascades initiated by mAb immunotherapy is shown in Figure 1. Further work must investigate the balance of stimulatory and immunosuppressive networks in conjunction with conventional chemo- and radiotherapeutic strategies. Incorporation of TA-targeted mAb should also be considered to a much greater extent in cancer vaccine-based strategies.[12,16,78]

A number of intriguing questions are raised by these data. For instance, if our hypothesis is correct, that TA-specific T-cell immunity plays an important role in the clinical responses to mAb-based immunotherapy, why are mAbs more effective than vaccines that elicit T

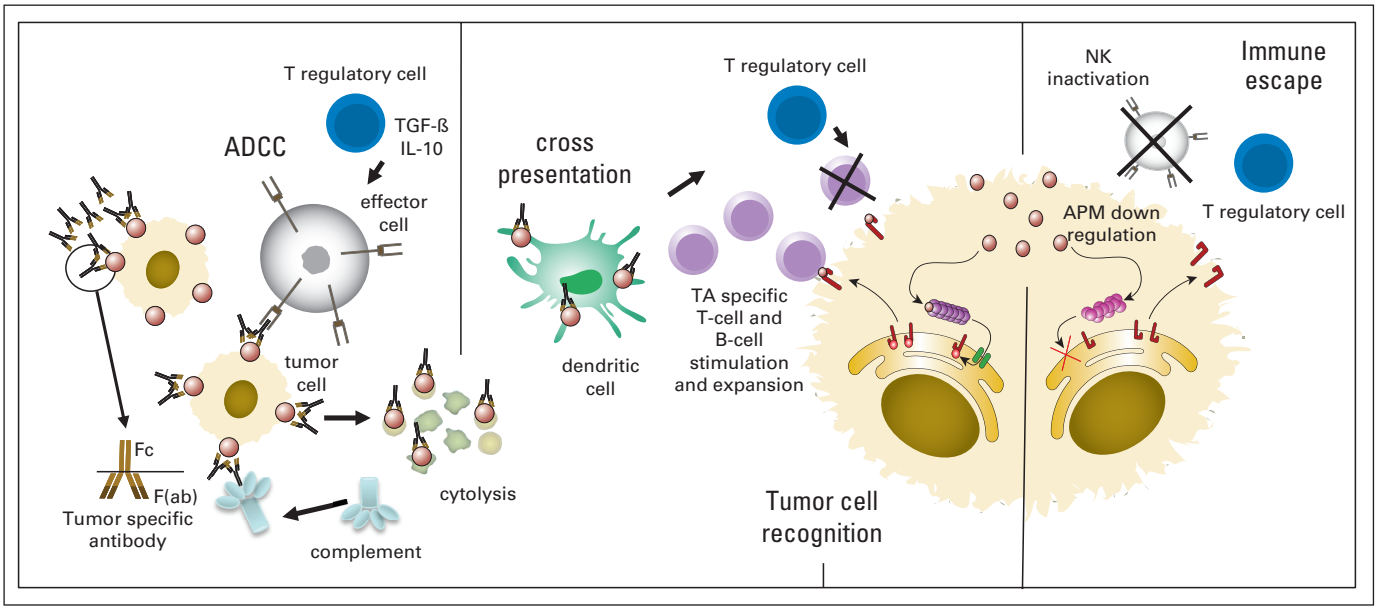

Fig 1. Immune cellular network mediated by tumor antigen (TA) –targeted monoclonal antibodies (mAbs) in the tumor microenvironment to induce antitumor activity. Direct cell lysis of mAb-bound tumor cells overexpressing the targeted TA may occur. First, exposure of TA-positive tumor cells to TA-targeted mAbs leads to their opsonization through the binding of cetuximab to the TA epitopes expressed on tumor targets. Recognition of tumor cells opsonized with mAbs is mediated via the FcγRIII (CD16) expressed on natural killer (NK) cells and FcγRIIa on monocytes, dendritic cells (DCs), and other granulocytes.[40,48] These effectors of innate immunity are activated in the presence of mAb-coated tumor cell targets and proceed to release perforin and granzymes, thus inducing tumor cell death (antibody-dependent cell-mediated cytotoxicity [ADCC]). The mAb-coated TAs released by dying cells in the form of immune complexes are avidly taken up by DCs, processed, and presented to T cells. This recruitment of NK cells and other FcγR-bearing immune cells occurs, liberating tumor cell products and TAs in the setting of inflammatory cytokines and chemokines. Infiltration of DCs and lymphocytes into the microenvironment may lead to uptake and processing of TAs by DCs and induction of TA-specific cellular immune responses. TGF-β, transforming growth factor β; IL-10, interleukin 10; APM, antigen processing machinery.

cells? This difference may reflect the induction by TA-targeted mAbs of a cellular immune response to a broader range of TA, including private antigens and cytokines in the tumor microenvironment. In addition, Th1 type cytokines may upregulate APM components in tumor cells and/or downregulate HLA-G, therefore counteracting escape mechanisms. If induction of a T-cell response is important, the combination of TA-specific mAbs with vaccination or immunomodulators such as anti-CTLA4 mAb garners greater rationale. The latter may be more effective because we have not yet identified the TAs that are clinically relevant. Patients treated with TA-targeted mAbs may also be a useful source of T cells to identify new, clinically relevant TAs. Downstream immunologic effects, such as the triggering of the idiotypic cascade, which we have observed in cetuximab-treated patients with HNC (unpublished data), is an area for further investigation. Finally, passive administration of mAb might be replaced by vaccination with peptide mimics to induce TA-targeted antibodies, as recently described in the HER2 system.[119]

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Glossary Terms

ADCC (antibody-dependent cell-mediated cytotoxicity): a mechanism of cell-mediated immunity whereby an effector cell of the immune system actively lyses a target cell that has been bound by specific antibodies.

Antigen processing machinery: a pathway of degradative and chaperone proteins in the cytoplasm and endoplasmic reticulum (ER) that process and transport antigen, degrading whole protein in the cytoplasm into short peptide fragments, which are transported into the ER and then bound to HLA antigens. The trimolecular HLA-β2 microglobulin-antigenic peptide complex is then transported to the cell surface for presentation to T lymphocytes.

CDC (complement-dependent lysis): process of target cell lysis by a cascade of soluble proteins activated by cells coated with immunoglobulin G or immunoglobulin GM antibodies.

Cross-presentation: a process of antigen-specific T-cell stimulation by dendritic cells and other antigen-presenting cells, which take up exogenous antigen and process it for recognition by HLA class I-restricted cytotoxic T lymphocytes, and in some cases HLA class II-restricted antigen presentation to CD4+ T lymphocytes.

Cytokines: Cell communication molecules that are secreted in response to external stimuli.

Cytotoxic T lymphocyte: a T lymphocyte (a type of white blood cell) that is capable of inducing the death of tumor cells; they also kill cells that are infected with viruses.

Immunoescape: a general term referring to the many efforts by tumor cells to suppress or evade antitumor immunity. This may lead to upregulation of inhibitory proteins, or downregulation of required proteins necessary for efficient antitumor immunity.

Microenvironment: the unique complex of tumor cells, stromal, and immune infiltrate that can promote or reject tumors, as well as shape their phenotype through contact-dependent or soluble mediators.

NK cells (natural killer cells): NK cells belong to the innate immune system and are specialized to kill target cells that are either infected with viruses or host cells that have become cancerous. CD56 is a surface marker specific to NK cells.

Regulatory T cells (known as suppressor T cells): are a specialized subpopulation of T cells that act to suppress activation of the immune system and thereby maintain immune system homeostasis and tolerance to self-antigens. This is an important "self-check" built into the immune system so that responses do not go haywire. Regulatory T cells come in many forms, including those that express the CD8 transmembrane glycoprotein (CD8+ T cells), those that express CD4, CD25 and Foxp3 (CD4+CD25+ regulatory T cells or "Tregs") and other T cell types that have suppressive function. These cells are involved in closing down immune responses after they have successfully tackled invading organisms and also in keeping in check immune responses that may potentially attack one's own tissues (autoimmunity).