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Tumor antigen-specific monoclonal antibodies and induction of T cell immunity

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Abstract

For decades the primary available cancer therapies were relatively nonspecific cytotoxic agents which, while effective in some patients, were limited by narrow therapeutic indices, extensive toxicity and development of resistance, likely due to tumor heterogeneity. Although these chemotherapies remain common tools of conventional treatment, the approval of a growing number of tumor antigen (TA) specific monoclonal antibodies (mAb) by the FDA, has driven a shift in the paradigm of cancer therapy. For a subset of patients with lymphoma, colorectal, head and neck and breast cancer, the inclusion of rituximab (anti-CD20), cetuximab (anti-human epidermal growth factor 1), and trastuzumab (anti-human epidermal growth factor2) has resulted in overall improved clinical response rates and survival advantages. The mechanisms which contribute to these effects are not only limited to inhibition of signaling pathways, but also by cell-mediated cytotoxicity by innate immune cells and priming of effector cells of adoptive immunity triggered by the TA-specific mAb. As the use of these therapeutic mAb has become more widespread, however, it has been observed that there is significant variability of response in patients who have received treatment with these agents. Thus, the factors which mediate this variability in clinical response of the treated patients must be elucidated, in order to optimize the use of TA-specific mAb.

Introduction

The advent of tumor antigen (TA)-specific monoclonal antibodies (mAb) represented a major breakthrough in cancer therapy which, until that point, had been limited to broadly applied, non-tumor selective, cytotoxic agents. For a subset of patients these mAb confer an overall improved response to therapy and improved clinical outcomes [1]. In 1997 rituximab (anti-CD20) was one of the first TA-specific mAbs to receive FDA approval for use in the

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therapy for B-cell non-Hodgkin's lymphoma by the FDA. Since then, several TA-specific mAbs have received approval, notably, rituximab, (anti-human epidermal growth factor/HER2) for metastatic breast and gastric cancer, and cetuximab (anti-human epidermal growth factor 1) for colorectal and head and neck cancers. As a result of the interest in these mAb, patients have been recruited to clinical trials where more focused studies continue to be conducted in an attempt to characterize the effects of these drugs. Interestingly, it has been noted that these antibodies, whilst effective in up to 30% of patients when used in combination therapy [2, 3], show limited effectiveness in some patients, regardless of expression of the targeted TA on tumor cells. Here we examine the immunological processes which are affected by these mAb, the effector cells which mediate their results and the factors that continue to challenge their widespread use.

Mechanism of action of TA-targeted mAb

Trastuzumab is a humanized anti-HER2 IgG1 mAb which is effective in combination therapy for HER2 overexpressing breast cancers and metastatic gastric or gastroesophageal junction adenocarcinomas [4]. HER2 overexpression occurs in 15-20% of patients with breast cancer [5], of these patients, 25-30% respond to trastuzumab [6]. Rituximab is a chimeric human-murine IgG1 mAb targeting CD20 receptors which are expressed on malignant B cells. It is effective in therapy for B cell malignancies including chronic lymphocytic leukemia (and CLL) non-Hodgkin's lymphoma (NHL) [7, 8]. Cetuximab, the chimeric human-murine IgG1 mAb was developed to target human epidermal growth factor 1 receptor (EGFR) which is overexpressed on multiple epithelial cancers. Initially approved for use in malignant colorectal carcinoma [9, 10], its use has now been extended to head and neck squamous cell carcinoma [11, 12]. The mechanisms of action of these antibodies consist of several processes. The cell surface targets for these antibodies are expressed on both healthy and malignant cells, although in higher concentration in cancer cells compared to healthy [13]. Binding of these mAbs to their cognate receptors results in blockade of downstream signal transduction and subsequent inhibition of proliferative, survival and anti-apoptotic pathways. HER2 and EGFR are tyrosine kinase receptors which signal through the phosphatidylinositol 3-kinase (PI3K)/AKT and Ras/mitogen-activated protein (MAP) kinase pathways [14]. Activation of CD20 triggers anti-apoptotic pathways through Bcl-2 in B cells [15]. Levels of expression of these tumor antigens have not been correlated with clinical outcomes for the patient supporting an argument that although inhibition of signal transduction plays a role in the therapeutic efficacy of these mAbs, there are other, immunological mechanisms which add to their function [16, 17]. Indeed, without the addition of immune effector cells to the in vitro system, apoptosis of tumor cells are not induced by these mAbs alone [18]. These immune mechanisms include antibody-dependent, cell-mediated cytotoxicity (ADCC), complement mediated cytotoxicity [19] and adaptive immunity mediated by induction of CD8⁺ cytotoxic T lymphocytes.

Antibody-dependent cell-mediated cytotoxicity (ADCC)

Antibody-dependent, cell-mediated cytotoxicity (ADCC) is an important lytic reaction which facilitates the anti-tumor mechanism. TA-specific mAbs coat tumor antigen and bind to Fc γ receptors (Fc γ R) expressed on NK cells, antigen presenting cells or directly on

immune effector cells causing them to become active and lyse the antibody coated tumor cell [20]. The importance of this reaction has been shown in *in vivo* studies as well as studies in FcγR –deficient murine models [21, 22]. IgG1 mAbs including rituximab, trastuzumab and cetuximab have all been shown to exert their anti-tumor effects, at least in part, through NK-cell mediated ADCC [23-26]. The variables which can alter the efficacy of ADCC have been extensively investigated, the most prominent of these, FcγR polymorphisms, has shown some contradictory results [27].

FcγR Polymorphisms

Three classes of FcγR encoded by 8 genes located in the same region of the long arm of chromosome 1 have been identified, (FcγRI/CD64, FcγRII/CD32 and FcγRIII/CD16) [28] FcγRIIIa are expressed on NK cells while both FcγRIIIa and FcγRIIa are expressed on antigen presenting cells. Some FcγR contain single nucleotide polymorphisms (SNPs) within coding regions of their genes which result in the generation of allotypes which can alter their function in the ADCC mechanism and subsequently, responses to TA-specific mAbs [27]. First described in rituximab therapy for lymphoma, the H131R coding polymorphism in the extracellular domain of FcγRIIa describes the amino acid substitution at position 131 changes from histidine to arginine [29]. The resulting R/R genotype is found to bind human IgG1 with the same affinity but interestingly, does not appear to bind human IgG2 well [30, 31]. Another FcγR polymorphism occurs in the FcγRIIIa where valine is substituted for phenylalanine at position 158 -V158F, with the V allele having a stronger binding affinity for human IgG1 [32, 33].

The clinical relevance of this polymorphism is under some debate and appears have variable significance in therapy with TA-specific mAbs. In pre-clinical studies looking at trastuzumab mediated ADCC there was a trend towards the FcγRIIa H/H and FcγRIIIa V/V genotypes and improved ADCC [21]. Similar trends were observed in clinical outcomes for patients treated with trastuzumab in smaller studies [6, 34]. In fact, the combination of FcγRIIa H/H and FcγRIIIa V/V genotypes appeared to compound their positive outcomes [6]. However, in a large study which examined patients treated with early-stage as well as metastatic HER2- positive breast cancer, the correlation between FcγR genotype and clinical outcomes measures was not statistically significant. In the case of single-agent rituximab therapy, initial studies reported higher rituximab response rates associated with the FcγRIIa H/H and FcγRIIIa V/V genotypes [29, 35]. Conversely, more recent studies have suggested that FcγR genotype is not significantly associated with long term clinical outcomes [36, 37]. Cetuximab therapy for colorectal carcinoma is noteworthy because some studies show increased response to therapy is associated with both the FcγRIIIa V/V [38, 39] and F/F [40] genotypes. In vitro studies looking at cetuximab therapy for head and neck cancer indicate a correlation with the FcγRIIIa VV genotype and cetuximab-mediated ADCC [41]. Data from a retrospective single-agent cetuximab trial showed a lack of correlation between clinical outcome and these alleles [42], which was confirmed using specimens from the Radiation Therapy Oncology Group (RTOG) 0522 study, a large randomized phase III trial of concurrent radiation and cisplatin therapy versus radiation, cisplatin, and cetuximab on patients with stage III and IV head and neck carcinomas (ASTRO proceedings 2014). However, no cetuximab alone arm existed on this trial, raising the question whether the

concurrent use of cisplatin with cetuximab may have confounded this lack of correlation with clinical outcome.

NK-DC Crosstalk

Although dendritic cells (DC) typically present endogenous peptides via the HLA-class I complex, they play a crucial role in anti-tumor responses as they prime naive T cells with tumor antigen as well as providing co-stimulatory signals which activate the T cell and enable lysis of malignant cells [43-45]. This function, where DCs can present non-endogenous peptides using HLA- class I complexes is described as cross presentation [46]. Additionally, DCs carry an additional signal (signal 3) which drives T cell responses towards either a TH1 or TH2 direction. Natural killer (NK) cells interact with DCs in a bidirectional manner, with both NK and DCs being capable of activating each other [47-49]. This interaction is dependent on the activating receptor NKG2D found on NK cells, which is necessary for appropriate NK cell activation and DC maturation [26]. TA-specific mAbs have been shown to activate NK-DC crosstalk thus facilitating improved T cell responses [50, 51]. Evidence suggest that NK cells continue to have a role in facilitating CD8+ T cell responses both *in vitro* and *in vivo* as well as supporting DC maturation [52, 53]. Interestingly, FcγRIIIa polymorphisms do not appear to correlate with cetuximab induced NK-DC crosstalk or DC maturation both *in vitro* and *in vivo* (Lord et al unpublished data). Pre-clinical studies using cetuximab treated DCs in head and neck squamous cell carcinoma cell lines reveal that this mAb can both enhance cross presentation of TA and skew the tumor microenvironment towards an milieu which promotes priming of T cells [26]. Additionally, clinical studies in patients with breast cancer treated with trastuzumab have shown improved CD4+ T cell responses supporting the idea that mAbs enhance cellular responses [54].

Augmentation of T cell priming by DC

One of the primary actions of TA-specific mAbs is enhancement of antigen cross presentation by mature DCs to T cells both *in vitro* and *in vivo*. This is important as it leads to the generation of CTL which are primed to target tumor antigen on malignant cells. Naive T cells routinely circulate between the peripheral blood and lymph nodes, if the T cell receptor on these cells recognize antigen presented on the major histocompatibility complex (MHC) with co-stimulation, they can become primed and activate against the antigen target [55]. Evidence in murine models of breast cancer treated with trastuzumab shows that addition of this mAb enhances priming of CD8+T cells by DC [56, 57]. *In vitro* studies looking at cetuximab used for the treatment of colorectal carcinoma also resulted in the generation of cytotoxic T lymphocytes with effective anti-tumor activity [58]. In addition to T cell priming, TA-specific antibodies augment the release of T-helper cell 1 (Th1) cytokines. Th1 cytokines are responsible for pro-inflammatory responses which are critical for cellular immunity. The primary Th1 cytokine is Interferon gamma (IFNγ) [59]. One study looking at cetuximab treated head and neck cancer cell lines which were co-cultured with NK and DC cells demonstrated an increase in secretion of multiple Th1 cytokines over cells untreated cells [42]. As yet results from *in vivo* studies using these antibodies are not

available but it is hoped that enhanced mAb enhanced T cell priming will result in better clinical outcomes for the patient.

Murine models have provided evidence that the stability of DC – T cell interaction is crucial for development of effective CD8+ T cell responses, in fact, there is an association between unstable, intermittent DC-T cell interactions and the development of regulatory T cells and T cell tolerance [60, 61]. T cell tolerance and the development of regulatory T cells is an area of interest in anti-tumor therapy, the mechanisms which bring about this phenomena are varied and still being understood.

Human Leukocyte Antigen (HLA)-Class I the antigen processing machinery (APM)

The antigen processing machinery consists of a complex system of intra and extracellular components which generate HLA-class I TA-derived peptides for expression by antigen presenting cells [62, 63]. In tumor cells and antigen presenting cells cross-presenting tumor antigen, the HLA-class I pathway mostly processes endogenous antigens which are then presented on the cell surface and recognized by the equivalent T cell receptor on T cells [64, 65]. Correct functioning of all components of the APM is necessary for this system to function precisely, abnormalities in expression or function of APM components are seen in 50-70% of malignancies and constitutes an important mechanism by which the tumor evades the immune system [62, 66, 67] This represents a mechanism of potential mechanism by which tumors may escape from mAb induced adaptive immunity. In HER2 overexpressing breast cancers, studies have shown that tumor escape from the host immune system may be a result of MHC class I downregulation by HER2 overexpression [68-70]. Similar results are seen in squamous cell carcinoma of the head and neck where EGFR overexpression correlates with downregulation of MHC class I [67, 71]. As a consequence of impaired tumor antigen processing and presentation, there is poor recognition of tumor cells by cytotoxic T lymphocytes and the development of an immune escape phenotype which correlates with poor clinical outcome [66, 72].

Regulatory T cells (Treg)

This important subpopulation of T cells acts to suppress the immune system and is believed to have developed as an internal regulation to prevent aberrant or excessive immune responses [61, 73]. The name Tregs is given to the form of regulatory T cells which express CD25, CD4 and the transcription factor, Foxp3 [74]. There are at least 3 classes of Tregs described in humans, CD4+CD25+Foxp3+ Tregs, Th1 Tregs, and Th3 Tregs [75]. The development of regulatory T cells can be detrimental to anti-tumor immunotherapies and the factors which lead to their expansion are currently under investigation. Within the tumor microenvironment, Tregs may result from chronic exposure of T cells to self –antigens, including those which are used as TA-specific mAb targets, such as EGFR [76]. For example, although CD8+ T cells are present in some patients with head and neck squamous cell cancer, their anti-tumor activity may be suppressed by Tregs thus the tumor remains unchecked [77]. Higher numbers of Tregs are seen in peripheral blood of patients with cancer compared to healthy controls and interestingly, many tumors have been shown to

inhibit the action of tumor infiltrating effector immune cells by recruiting Tregs to the tumor site [78-81]. Although these TA-specific cytotoxic T lymphocytes become inactive, it has been reported that these cells may in fact be in a state of latency and can be recovered to functioning T cells both *in vitro* and *in vivo* [82, 83]. In a trial looking at clinical samples from patients with head and neck cancer, tumor associated Tregs have been shown to express higher frequencies of immune checkpoint inhibitors than peripheral blood Tregs [84]. Immune checkpoint receptors (ICR) include programmed cell death-1 (PD-1), cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), lymphocyte activation gene-3 (LAG-3), and T-cell immunoglobulin and mucin protein-3 (TIM-3). Although it is not yet clear what role these ICR play in the generation and immunosuppressive function of Tregs, it is clear that they are key molecules involved in the generation and maintenance of Tregs [85, 86]. Interestingly, although there are greater number of Tregs in both the peripheral blood and tumors of patients with cancer compared to healthy donors, Tregs found in the tumor microenvironment appear to have a significantly more immunosuppressive phenotype than those found in the peripheral blood, which may be accounted for by elevated expression of ICR on their surface [84]. Recent data suggest mAb may promote increased Treg frequency and enhanced expression of ICR and other immunosuppressive chemokines. In a prospective trial of cetuximab treated patients with head and neck cancer, an increased frequency of intertumoral Tregs was observed, in addition, these Treg showed enhanced expression of CTLA-4, CD39 and TGF- β which correlated with poorer clinical outcomes for the patient (Jie, H-B et al, submitted).

Summary

Therapeutic, TA-specific mAb are important components of the therapeutic armamentarium used in cancer patients, but their actual mechanism(s) continue to be elucidated. This information is crucial to understanding variability in clinical response and biomarkers, which may facilitate selection of populations most likely to derive clinical benefit. In addition, rationale design of combination therapies with these mAb and other agents would be enhanced by a greater understanding of the antitumor activity observed in order to amplify tumor-specific immunity and mobilization of effector cells and long-lasting remission associated with immunologic memory.

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