Published in final edited form as:

Immunol Res. 2011 August ; 50(2-3): 248–254. doi:10.1007/s12026-011-8231-0.

Natural killer (NK):dendritic cell (DC) cross talk induced by therapeutic monoclonal antibody triggers tumor antigen-specific T cell immunity

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Abstract

Tumor antigen (TA)-targeted monoclonal antibodies (mAb), trastuzumab, cetuximab, panitumumab, and rituximab, have been among the most successful new therapies in the present generation. Clinical activity is observed as a single agent, or in combination with radiotherapy or chemotherapy, against metastatic colorectal cancer, head and neck cancer, breast cancer, and follicular lymphoma. However, the activity is seen only in a minority of patients. Thus, an intense need exists to define the mechanism of action of these immunoactive mAb. Here, we discuss some of the likely immunological events that occur in treated patients: antibody-dependent cellular cytotoxicity (ADCC), cross talk among immune cells including NK cells and dendritic cells (DCs), and generation of TA-specific T lymphocyte responses. We present evidence supporting the induction of “NK:DC cross talk,” leading to priming of TA-specific cellular immunity. These observations show that mAb-mediated NK cell activation can be greatly enhanced by the action of stimulatory cytokines and surface molecules on maturing DC and that NK:DC interaction facilitates the recruitment of both NK cells and DC to the tumor site(s). The cooperative, reciprocal stimulatory activity of both NK cells and DC can modulate both the innate immune response in the local tumor microenvironment and the adaptive immune response in secondary lymphoid organs. These events likely contribute to clinical activity, as well as provide a potential biomarker of response to mAb therapy.

Keywords

Antitumor monoclonal antibodies; Cellular immunity; Tumor antigens; ADCC; Immunotherapy

Introduction

Monoclonal Ab (mAb)-based therapy has demonstrated success against various malignancies, and an increasing number of such mAb are being included in cancer therapy [1]. However, tumor antigen (TA)-specific mAb therapy is successful only in a subset of patients. Although the benefits for mAb-based immunotherapy are currently limited, investigations designed to improve this type of therapy are underway. Various lines of evidence indicate that rituximab (anti-CD20), trastuzumab (anti-HER2), and cetuximab (anti-HER1/anti-EGFR) are used in lymphoma, breast cancer, head and neck, and colorectal carcinomas, respectively (Table 1), and the activities are enhanced by the combination of chemotherapy or radiotherapy [2–10]. Indeed, as a single agent, TA-targeted mAbs lead to only a 10% response rate in advanced, heavily pretreated and recurrent disease; nevertheless, the therapeutic success is enhanced to around 20% by combining radiotherapy or chemotherapy with mAb therapy [11]. Having limited success, various clinical reports emphasize the need to characterize the mechanism(s) underlying the antitumor activity of TA-specific mAb, which would permit the selection of patients that are most likely to benefit, and lead to potential augmentation strategies. Despite their direct effects on tumor cell signaling, these therapies must be incorporated into models reflecting the immunologic mechanism of action, including the effect on NK cells, DC, and T cells (Table 2).

CD20, EGFR (Erb1, HER1), and HER2 (Erb2) are overexpressed on malignant cells but not on normal cells and are targeted by the TA-specific mAb. In most cases, quantitative differences define the pathogenesis of malignancy, and a correlation between the overexpression of these molecules and worse prognosis has been reported [12]. CD20, the target receptor for rituximab, triggers antiapoptotic pathways in B cells through Bcl-2 [13, 14]. EGFR and HER2 are tyrosine kinase receptors that belong to Erb-b/HER receptor family. HER1 and HER2 initiate signaling through several pathways including PI3Kinase/AKT and Ras/MAP kinase pathway that promote cell survival and proliferation. Cetuximab blocks the tumor cell proliferation by interfering with the EGFR dimerization [1, 15]. Despite their likely direct, antiproliferative effects mediated by preventing ligand binding to their cognate surface receptor (EGFR, HER2, or CD20), we review here the immunological effects on NK cells and DC of antitumor mAb, leading to enhanced DC maturation and TA processing, ultimately resulting in priming of T cell-based immunity.

ADCC, Fc γ receptor affinity, and the efficacy of antitumor mAb

ADCC takes place upon interaction of Fc-region of mAb with immune cells, when the Fab region of the mAb binds to the antigenic epitope on tumor cells [16]. This interaction induces the activation of Fc γ R-bearing immune cells (granulocytes), leading to the destruction of tumor cells by the release of lytic granules from the effector immune cell. Lysed tumor cell (antigenic material) can then be engulfed by DC, a process that is enhanced when apoptotic TA-expressing cellular products are coated with mAb, which further generates the cellular cascade leading to antitumor immune responses (Fig. 1). Different isotypes of mAb bind to Fc γ RIIIa (expressed by NK cells) with highly variable affinity, which appear to be a determining factor for the efficacy of NK cell-mediated ADCC, but important downstream immunological responses are also possible when Fc γ RIIa (CD32) receptors are present, such as on DC and neutrophils. A polymorphism in human Fc γ RIIIa codon 158 also exists, and the clinical benefits of TA-specific mAbs seem

to be correlated with the homozygous patients who carry high-affinity Fc γ RIIIa allele—V (valine). The functional impact of this Fc γ RIIIa polymorphism has been observed in multiple malignancies by its association with the degree of lysis of tumor cell targets in vitro. This polymorphism (VV/VF/FF) greatly affects the binding affinity of mAb, particularly of the IgG1 isotype [1, 17–19]. The direct in vivo relevance of such affinity-based differential ADCC has not yet been validated prospectively and is based on retrospective, correlative cohort studies. The commonly occurring IgG1 isotype mAb, such as rituximab, trastuzumab, and cetuximab, shows differential NK cell-mediated ADCC, based on the affinity to the VV, VF, or FF genotype [17]. Uniquely, panitumumab (IgG2 isotype, fully human mAb) is specific for EGFR, similar to cetuximab, providing an interesting in vitro model to probe immunologic effects that can also be evaluated in treated cancer patients since both mAbs are now FDA approved. Upon targeting two distinct isotype classes of mAb (panitumumab:IgG2; cetuximab:IgG1) that bind to EGFR, the enhanced level of activation of NK cells was only seen with cetuximab but not with panitumumab [20]. No published data exist that panitumumab clinical activity correlates with Fc γ RIIIa codon 158 (V/F) polymorphism, and its interaction appears mainly to occur with Fc γ RIIa, perhaps expressed by monocytes or neutrophils. However, a recent study showed the ability of panitumumab to mediate ADCC through other myeloid/granulocytic cells [22]. Further studies are being conducted in various laboratories to investigate whether ADCC through myeloid cells is an actual mode of action of panitumumab in vivo.

Along with the affinity, the concentration, association constant of mAb play a crucial role in the cytotoxicity [1]. The dose–response relationship for cetuximab against HNC and lung cancer cell has been established in vitro. The dose above 10 μ g/ml shows saturation in the binding and ADCC, which indicate abundant amount of mAb in vivo in circulation [1, 20, 21]. This result also shows the affinity-based interaction of IgG to the Fc γ R. The resulting activation through cetuximab-activated NK cells and DC collaborate to trigger tumor antigen-specific T cell immunity in head and neck cancer patients (unpublished data).

At this point, a similar correlation for the effect of Fc γ RIIa or IIIa genotype has not been demonstrated for mAb-activated, NK cell-dependent DC maturation (Figs. 2, 3) or T cell priming. Other molecular factors may also mediate NK cell interactions, including reciprocal stimulation in collaboration with the DC network in the tumor microenvironment, which then promotes NK cell cytokine secretion, DC maturation, and TA cross-presentation to prime T cell responses.

TA-specific mAbs initiate NK:DC cross talk leading to cellular immunity

Various reports indicate that antitumor mAb can generate T cell-based cellular immunity [1, 16]. Dhodhapkar et al. reported that the coating of myeloma cells with tumor-reactive antisyndecan-1 mAb promoted the cross-presentation of TA by DC to T cells. They also reported that the enhanced cross-presentation of TA was dependent on the Fc γ R, but was not dependent on the uptake of tumor cell material [23]. Dhodhapkar's group also showed that the blocking of inhibitory Fc γ RIIb on DC that were loaded with tumor cells also generated T cell immunity in the presence of plasma. These finding also revealed the role of the balance of activating and inhibitory Fc γ R on the cross-presentation of TA [24]. Selective targeting the cetuximab-coated glioma cell to DC also generated T cell-specific immune responses. In this process, the targeted activating Fc γ R played a critical role without any exogenous maturation stimuli [25]. The balance between the contrasting function of activating and inhibitory Fc γ RII was shown to govern the DC activation along with the uptake of immune complexes. The opposing effect of these activating and inhibitory Fc γ R appears to play a potential role in the pathophysiology of immune complex-mediated diseases [26].

The activity of anti-HER2/Erb-2 mAb, trastuzumab, used for the treatment of breast carcinoma, was recently reported to be dependent on the release of type I and type II IFNs but not on perforin or CD95 ligand. This study disregarded ADCC as the mechanism of action of this mAb, instead showed that trastuzumab therapy induces MyD88 signaling and primes IFN- γ -producing CD8⁺T cells [27]. Also, Knutson and Clynes have demonstrated the enhancement of HER-2 specific CD4⁺T cell responses in trastuzumab-treated breast carcinoma patients [28].

In addition to TA uptake and processing, T cell-based antitumor immune responses are dependent on DC receptors and maturation phenotype [29]. Along with the uptake of antigenic complexes, Fc γ R also modulate the DC maturation status, and the balance between the activating and inhibitory Fc γ R greatly influence the outcome of immunological responses. Ravetch's group showed that one class of Fc γ R interaction generates pro-inflammatory effects with immune complex, whereas therapeutic intravenous gamma globulin and its Fc fragments are anti-inflammatory. They also showed that the contrasting features of IgG are the result of differential sialylation of the Fc fragment. IgG could acquire anti-inflammatory properties because of Fc—sialylation. This differential sialylation may thus modulate the immunological responses against antigens [30]. A recent report showed that the IgG1 backbone (present on most FDA-approved therapeutic, TA-specific mAbs in the clinic) cross-linked and activated Fc γ RIIIa-bearing NK cells, which could enhance the maturation of co-cultured DC, along with the upregulation of activating NK cell molecules like CD25, CD69, NKp44, NKp30, and CD40 ligand. These Fc γ RIIIa cross-linked NK cells upregulated the expression of HLA-DR, CD86, and CD83 on co-cultured DC. These maturation markers on DC are well known to induce the activation of T cells [31].

DC incubated with cetuximab-stimulated NK cells facilitates the reciprocal, enhanced activation of NK cells (NK:DC cross talk)

Several reports indicate that the mechanisms of enhanced cross-presentation of TA by mAb treatment include enhanced uptake of TA:mAb complex by antigen-presenting cells, appropriate activating Fc γ R ligation and stimulation of DC, upregulation of costimulatory and adhesion molecules on the DC surface, as well as upregulation of APM components, which is known to contribute the optimal TA processing and presentation [1, 16, 32].

Cetuximab treatment of NK cells in the presence of PCI-15B HNC cells enhanced the level of intracellular IFN- γ in NK cells. IFN- γ -activated DC furthermore reciprocally stimulated NK cells, leading to the increased secretion of IFN- γ . An isotype control IgG1 mAb or anti-EGFR, IgG2 isotype mAb panitumumab did not induce the activation of NK cells, as indicated in the respective conditions (Fig. 2). The reciprocal activation of cetuximab-activated NK cells and autologous DC is likely responsible for contributing to a DC-1 phenotype and Th1 cytokines/ chemokines in the microenvironment, enhancing DC processing of TA released by dying tumor cells and TA-specific T cell priming (Figs. 2, 3).

We have recently identified that the interaction of cetuximab with EGFR on tumor cells and Fc γ R IIIa on NK cells enhances cross-presentation of TA, such as EGFR, by DC to cytotoxic T lymphocytes (CTL) (Fig. 3). This effect is likely to reflect the upregulation by cetuximab of DC maturation markers and antigen-presentation machinery (APM) components, such as TAP-1/2 [32]. The clinical relevance of the in vivo effect of cetuximab is indicated by the significantly higher number of circulating EGFR_{853–861}- specific T cells in cetuximab-treated, HLA-A2⁺ HNC patients than in cetuximab-naïve HNC patients (unpublished data). We have also observed that non-EGFR TA can be transferred efficiently to local DC, enhanced by cetuximab-activated NK cells, resulting in priming of “private” TA specific to individual tumor cells. Therefore, cetuximab administration triggers a TA-

specific cellular immune response, suggesting a novel potential mechanism for its clinical efficacy. This information may contribute to optimize the selection of patients to be treated with this type of mAb-based therapy.

Cetuximab enhances cross-presentation of TA by DC in the presence of NK cells

To investigate whether cetuximab could induce EGFR-specific cellular immunity, we studied its effect on DC cross-presentation using EGFR⁺ HLA-A2⁻ HNC cells to stimulate EGFR-specific CTL. First, HLA-A2⁺ DC was incubated with cetuximab and the HLA-A2⁻, live PCI-15B HNC. The cross-presentation of EGFR₈₅₃₋₈₆₁-specific CTL with stimulated HLA-A2⁺ DC was quantified by IFN- γ ELISPOT assays. DC incubated with PCI-15B HNC and cetuximab significantly enhanced ($P = 0.001$) the cross-presentation of PCI-15B HNC cellular antigen to EGFR-specific CTL in the presence of NK cells. At basal level, the cross-presentation was observed alone by PCI-15B HNC without cetuximab, using a control IgG1 mAb or without NK cells (Fig. 3). Irrelevant specificity, control IgG1 isotype or IgG2 anti-EGFR mAb panitumumab failed to facilitate the enhancement of cross-presentation of these distinct TA in the presence or absence of NK cells. The findings presented above could reflect targeting of PCI-15B HNC to Fc γ R on DC by cetuximab, leading to enhanced EGFR uptake, processing, and presentation. To investigate this possibility, we also analyzed the rate of uptake of fluorescent PCI-15B HNC using flow cytometry after incubation with DC. However, no differences were observed in the uptake of either live or apoptotic tumor cells, in the presence of NK cells, cetuximab, or both (unpublished data).

NK:DC cross talk skews the tumor microenvironment toward a type 1 DC and T cell milieu

Although most innate immune cells express both activating and inhibitory Fc γ R, NK cells constitutively express only a low-affinity, activating Fc γ RIIIa. Fc γ RIIIa receptor upon encountering mAb-coated targets signals to initiate the secretion of cytokines and lytic activity. In addition to their ability to mediate ADCC, activated NK cells, particularly CD56^{bright} NK cells [1, 17, 33], which secrete T helper type 1 (Th1) cytokines, such as IFN- γ , TNF- α , and chemokines, such as macrophage inflammatory protein-(MIP)-1 α , MIP-1 β , RANTES, MCP-1 and IP-10 that inhibit tumor cell proliferation, enhance antigen presentation and facilitate chemotaxis of T cells [17, 34–37]. Various reports describe that the NK cell-mediated ADCC can be enhanced by the cytokines such as IL-21, IL-15, and IL-2. Triple-negative breast carcinoma cells that were resistant to the antiproliferative action of cetuximab showed high susceptibility with the IL-15-activated NK cells through ADCC [38]. In cancer patients, various reasons were described that paralyzes NK cell activity in vivo. Recently, IL-21 was shown to restore the killing potential of NK cells in HNC patients. Also, it was reported that IL-21 leads to the upregulation of CD247 on NK cells [39]. Another study described that cetuximab in conjunction with IL-2 can enhance ADCC against gastric cancer cells [40]. These observations concluded that NK cell-mediated ADCC and IFN- γ secretion can be greatly enhanced by the action of stimulatory cytokines (Fig. 2). “NK:DC cross talk” allows the recruitment of both NK cells and DC to the inflammatory sites [37, 41]. The cooperative activity of both NK cell and DC can modulate both the innate immune response in the local microenvironment and the adaptive immune response in secondary lymphoid organs. Specifically, NK cells in the presence of cytokines released by DC such as IL-12 become activated, regulating both the quality and the intensity of cellular immune responses. In turn, DC, in the presence of cytokines released by activated NK cells, such as IFN- γ , enhances cross-presentation and priming of T cells.

Thus, through direct interactions and secretion of cyto-kines/chemokines [17, 36, 37], NK cells may function as helper cells and enhance and broaden T cell priming against multiple TA. In an elegant study, the IL-18-dependent helper function of NK cell was described. Such CD56⁺/CD83⁺/CCR7⁺/CD25⁺ helper NK cells promoted IL-12 production in DC that may develop Th1 responses. Interestingly, PGE2 ablated the IL-18-induced helper function of NK cells [42, 43]. Brady et al. showed that the exposure of NK cells to IL-4, IL-12, IL-15, IL-18, and IL-21 induces NK cell maturation characterized by reduced proliferation and enhanced IFN- γ secretion. However, distinct combinations of cytokines induced different cytokine profiles. The immunosuppressive cytokines, IL-10 and TGF- β , have a negative impact on the function of NK cells. This report described that the cytokine milieu at the inflammatory site may greatly affect the function of NK cells [44]. Further studies specially examining the influence of mAb activation on these phenotypes and cell population are warranted.

Conclusions

The generation of TA-specific adaptive immune responses may offer substantial clinical benefit in response to treatment of cancer patients with therapeutic mAb. Indeed, mAb-based cancer therapy has very low side effects, similar to pharmacological inhibitors, but with the added benefit of TA-specific T cell priming and immunologic memory. In addition to their antiproliferative action against tumor cells, therapeutic mAb appears to trigger various immunological responses that may be responsible for their clinical activity. This cascade may be triggered by mAb-mediated ADCC, leading to the generation of a large amount of released TA, which can be engulfed, processed, and presented by DC or other antigen-presenting cells in vivo. The high pharmacokinetic stability of mAbs in the sera of patients and robust penetration of mAb to the tumor site are additional advantages. Moreover, various modes of engineering or modifying mAb structural regions may enable improvement in clinical activity. In addition, mAb therapy may synergize with other types of immunotherapy, such as DC-based vaccine strategies, and combinatorial therapy including mAb and radiotherapy or chemotherapies, which have already proven clinical benefit and induce antigen release through tumor cell lysis. Although generation of T cell immunity through NK:DC cross talk has been increasingly appreciated, it is indeed interesting to investigate whether mAb generate long-lasting central memory. Moreover, it is very important to clearly identify the immune modulators of the tumor microenvironment, such as specific cytokines or chemokines, which participate in the particular mAb-based immunological responses.

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Fig. 1.

NK:DC cross talk promotes tumor antigen cross-presentation and T cell priming. An antitumor mAb (cetuximab) binds to TA (EGFR) on tumor cell surface, whereas Fc region of mAb binds to Fc γ R on immune cells (NK cells). This event activates NK cells leading to the lysis of tumor cells that generates TA and TA:mAb complex. The tumor antigenic materials are engulfed by immature DC and TA are processed and presented by mature DC to generate TA-specific T cells via MHC I through cross-presentation. Mature DC may furthermore activate NK cell reciprocally. Moreover, cytokines secreted by mAb-activated NK cells during ADCC may provide additional maturation inducing signals to DC

Fig. 2. NK:DC interaction enhances cetuximab-mediated activation of NK cells. Measurement of activated NK cells incubated with PCI-15B HNC or DC and PCI-15B HNC in the presence of control IgG1 mAb, panitumumab, or cetuximab (each 10 μ g/ml) for 12 h by IFN- γ ELISPOT assay

Fig. 3.

Cetuximab enhanced the cross-priming of EGFR-specific CTLs in the presence of NK cells. EGFR₈₅₃₋₈₆₁ antigen-specific CTLs were stimulated with DC (from HLA-A2⁺ healthy donor) fed with EGFR-positive, HLA-A2⁻ live PCI-15B HNC (Tu) either with cetuximab or isotype-matched control IgG1 (each at 10 μ g/ml), with or without autologous NK cells (DC:NK: PCI-15B;1:1:1 ratio) for 48 h. Afterward, DC was incubated with naïve CD8 T cells for 36 h, and the frequency of EGFR₈₅₃₋₈₆₁ antigen-specific CTLs activated under indicated conditions were examined for IFN- γ production by ELISPOT

Table 1

TA-specific FDA-approved antitumor mAb against cancers in human

ImAb	Isotype class	Target molecule	Cancer types
Cetuximab	Chimeric IgG1	EGFR/HER1	Metastatic colon cancer, head and neck cancer
Panitumumab	Human IgG2	EGFR/HER1	EGFR-positive colon cancer
Trastuzumab	Humanized IgG1	HER2/ <i>neu</i>	HER2/ <i>neu</i> -positive breast cancer
Rituximab	Chimeric IgG1	CD20	CD20-positive low-grade lymphoma, diffuse large B cell lymphoma, follicular lymphoma

Table 2

Potential immunological mechanisms of antitumor mAbs

Immune effectors cells	Mechanisms	Effects
NK cells, macrophages	ADCC, secretion of immunomodulatory cytokines like type I and type II IFNs	Generation of tumor antigenic materials, Reciprocal NK:DC activation, NK cell activation (MICA:NKG2D)
DC	Uptake of tumor antigenic material from live cells or dead cells, cross-presentation of tumor antigens, processing, and presentation of antigens via MHCI and MHCII, secretion of Th1-polarizing cytokine	NK cell activation (MICA:NKG2D) Antigen spreading, generation, and expansion of CD4-positive and CD8-positive T cells