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The Therapeutic Effect of Anti-HER2/neu Antibody Depends on Both Innate and Adaptive Immunity

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SUMMARY

Anti-HER2/neu antibody therapy is reported to mediate tumor regression by interrupting oncogenic signals and/or inducing FcR-mediated cytotoxicity. Here, we demonstrate that the mechanisms of tumor regression by this therapy also require the adaptive immune response. Activation of innate immunity and T cells, initiated by antibody treatment, was necessary. Intriguingly, the addition of chemotherapeutic drugs, although capable of enhancing the reduction of tumor burden, could abrogate antibody-initiated immunity leading to decreased resistance to rechallenge or earlier relapse. Increased influx of both innate and adaptive immune cells into the tumor microenvironment by a selected immunotherapy further enhanced subsequent antibody-induced immunity, leading to increased tumor eradication and resistance to rechallenge. This study proposes a model and strategy for anti-HER2/neu antibody-mediated tumor clearance.

INTRODUCTION

The human epidermal growth factor receptor 2 (HER2, HER2/neu, or ErbB-2) is overexpressed in 20%–30% of breast carcinomas and is associated with aggressive disease, a high recurrence rate, and reduced patient survival (Hudis, 2007; Kiessling et al., 2002; Meric-Bernstam and Hung, 2006; Slamon et al., 1987). The use of trastuzumab (Herceptin), a humanized monoclonal antibody that binds the extracellular, juxtamembranal domain of HER2, has proved to be an effective treatment in animal and human studies (Hudis, 2007; Moasser, 2007). Many groups have demonstrated that anti-HER2/neu antibody can efficiently stop or slow the growth of HER2/neu$^+$ tumors in vitro (Hudis, 2007; Kiessling et al., 2002; Meric-Bernstam and Hung, 2006). Growth inhibition is mainly due to the induction of G_1 cell cycle arrest and is closely tied to increased $p27^{Kip1}$ expression, and reduced cyclin E expression (Le et al., 2005; Mittendorf et al., 2010). In addition, antibody treatment was shown to inhibit the ability of tumor cells to repair damaged DNA (Pegram et al., 1999). The combination of antibody treatment with multiple chemotherapeutic agents showed additive and synergistic effects in in vitro studies and in vivo xenograft tumor models (Pegram et al., 1999; Pegram et al., 2004). As a result, interference with HER2 oncogenic signaling and increased

Significance

Although anti-HER2/neu antibody is an effective adjuvant therapy targeting HER2$^+$ breast cancers, relapse often occurs even after prolonged treatment. Current understanding holds that this antibody therapy interrupts oncogenic signals and induces FcR-mediated cytotoxicity. This study reveals that the therapeutic effect of anti-HER2/neu antibody treatment also depends on adaptive immunity. Furthermore, this study demonstrates an interesting antibody-mediated mechanism whereby danger signals are required to mobilize and activate innate cells and prime the adaptive immune system for increase tumor clearance. However, antibody-initiated tumor regression can be impaired by certain chemotherapy regimens. This study has important clinical impact because various chemotherapy drugs have been used before or after antibody treatment.

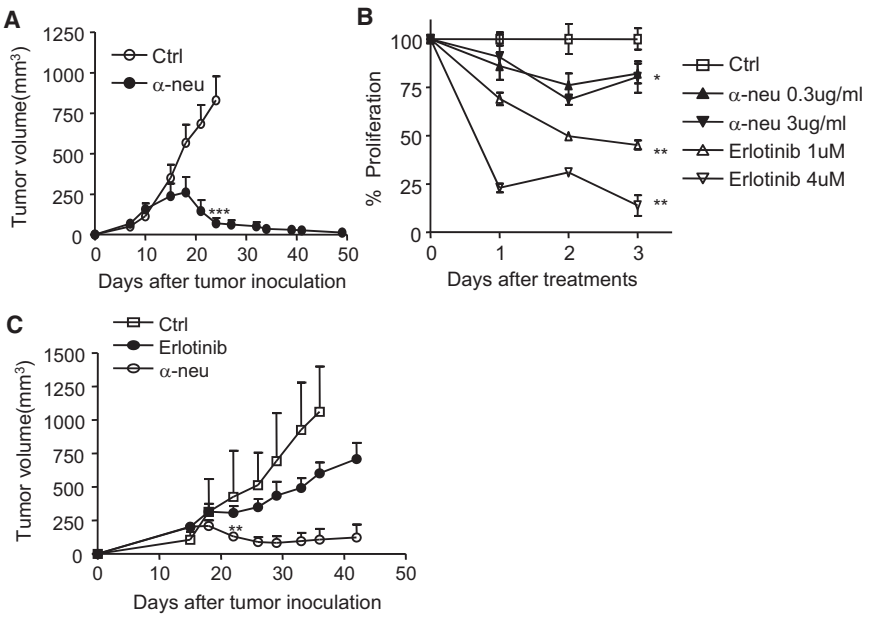

Figure 1. Anti-Neu Antibody Has Limited Effect In Vitro but Has Strong Effect Against Tumor In Vivo

(A) WT BALB/c mice (n = 5/group) were injected s.c. with 5 × 10^5 TUBO cells and treated with 100 μg of anti-neu (α-neu) or isotype control (Ctrl) antibody on days 14 and 21. The tumor growth was measured and compared twice a week. ***p < 0.005 compared with isotype control group after day 23. One of five representative experiments is shown.

(B) TUBO cells (1 × 10^5 cells/well) were plated in a monolayer and incubated with Erlotinib (1–4 μmol/l) or anti-neu antibody (0.3–3 μg/ml). Control groups received isotype-control antibody (Ctrl). Relative proliferation, reflected by metabolic activity, was evaluated at indicated times by MTT assay and graphed as percent of isotype control. Mean ± SD; *p < 0.05; **p < 0.005 compared with isotype control. One of two representative experiments is shown.

(C) TUBO-bearing BALB/c mice (n = 5/group) were treated four times with 100 μg of anti-neu antibody (α-neu) every other day and with 500 μg of Erlotinib every day for 7 days from day 18. *p < 0.05 compared with the control group from day 33; **p < 0.01 compared with erlotinib-treated group after day 26. One of two representative experiments is shown.

susceptibility to chemotherapy-induced apoptosis (chemosensitization) have been proposed as the central mechanisms responsible for the clinical efficacy of trastuzumab (Hudis, 2007; Moasser, 2007; Pegram et al., 2004). Based on the convincing preclinical studies, clinical trials were conducted and demonstrated the benefits of combining chemotherapy administration with trastuzumab (Hudis, 2007; Piccart-Gebhart et al., 2005; Romond et al., 2005). Despite of the initial clinical success of antibody plus chemotherapy treatment for $Her2^+$ tumors, relapse has been reported after cessation of this treatment.

Considering reports that inhibition of oncogenic signals by anti-HER2/neu antibody controls tumor growth in vitro, it was surprising that the therapeutic effect of this antibody was diminished in the absence of Fc receptor (FcR) signaling in vivo (Clynes et al., 2000). The role of FcRs in the efficacy of antibody treatment is further supported by evidence that *Fcr* polymorphisms are associated with the clinical outcome in breast cancer patients (Musolino et al., 2008). These data raise the possibility that antibody-dependent cellular cytotoxicity (ADCC) may play a major role in the antitumor effects of antibody therapy. Consistently, an increase of tumor-infiltrating leukocytes, especially FcR^+ cells such as NK cells, has been observed in tumor tissue after antibody treatment (Arnould et al., 2006; Varchetta et al., 2007). Furthermore, it was reported that patients with partial or complete remission after antibody treatment had higher in situ infiltration of leukocytes and an increased capacity to mediate in vitro ADCC activity (Gennari et al., 2004). Endogenous anti-HER2 antibodies after vaccine can be detected in some patients and can effectively suppress HER2 kinase activity and downstream signaling to inhibit the transformed phenotype of HER2-expressing tumor cells (Montgomery et al., 2005). However, most models, including xenografts used for preclinical evaluation, fail to account for adaptive immunity in the antibody-mediated therapeutic effect. Therefore, the essential role of T and B cells in anti-HER2/neu antibody-mediated tumor regression remains unclear.

RESULTS

Adaptive Immunity Is Essential for the Therapeutic Effect of Antibody Treatment

To evaluate whether targeted antibody treatment of $HER2/neu^+$ breast cancer could reduce tumor burden in syngeneic wild-type (Wt) mice, we used the well-characterized anti-neu (rat homolog of human HER2) monoclonal antibody 7.16.4 (Zhang et al., 1999). This antibody competes with 4D5 (the original mouse anti-HER2/neu antibody that was humanized to trastuzumab) for binding to human HER2 and inhibition of tumor growth. BALB/c mice bearing established TUBO tumors, a neu overexpressing cell line derived from a spontaneous carcinoma in neu-transgenic mice (Rovero et al., 2000), were treated with anti-neu antibody. Impressively, without the addition of chemotherapy, the majority (28/35) of Balb/c mice from several experiments rejected tumors completely 4 weeks after treatment, whereas control Ig-treated mice had to be sacrificed due to large tumor burden (Figure 1A and Table 1).

To test the relative contribution of HER2/neu signal interference with anti-neu antibody-mediated tumor regression, we compared the efficacy of this antibody to erlotinib, a tyrosine kinase inhibitor (TKI), in vitro and in vivo. A high dose of anti-neu antibody slightly inhibited TUBO cell proliferation in vitro (~20%), but effectively reversed the growth of or cleared all established tumors in vivo (35/35). On the other hand, erlotinib strongly inhibited TUBO cell proliferation in vitro (>80%) but