Antihuman Epidermal Growth Factor Receptor 2 (HER2) Monoclonal Antibody Trastuzumab Enhances Cytolytic Activity of Class I-restricted HER2-specific T Lymphocytes Against HER2-overexpressing Tumor Cells

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Abstract

The monoclonal antibody trastuzumab (Herceptin) directed against the human epidermal growth factor receptor 2 (HER2) results in tumor regressions when administered to patients with HER2-overexpressing breast cancer. One of the underlying mechanisms of this antibody-induced tumor regression is based on the internalization and degradation of HER2 by tumor cells on interaction with trastuzumab, subsequently inhibiting signal transduction pathways. As antibody-induced degradation of HER2 is likely to be accompanied with increased numbers of HER2 peptides presented with MHC, we asked whether trastuzumab-treated tumor cells were more susceptible to CTL-mediated lysis. Here we show that the cytolytic activity of human, HER2-specific CD8\(^+\) CTLs is augmented by anti-HER2 antibody trastuzumab. HER2-reactive CTL clones lyse class I-matched, HER2-overexpressing tumor cells more efficiently after treatment with trastuzumab. The potentially synergistic activity of HER2-specific antibody and CTL encourages the development of an HER2-targeted immunotherapy using a combination of inhibitory antibodies and CTLs for patients with HER2-overexpressing tumors.

Introduction

HER2\(^+\) represents an appealing target for humoral and cellular immunotherapy because HER2 is expressed at high levels in a variety of human cancers, such as breast cancer (1). Overexpression of antigenic proteins, such as HER2, is coupled with a high protein turnover, leading subsequently to a high number of MHC class I peptide complexes on the cell surface (Ref. 2; for review, see Ref. 3). Therefore, HER2\(^+\) tumor cells are potentially good target cells for tumor-reactive CTLs recognizing HER2-derived peptides in context with MHC class I molecules. Indeed, HER2-specific CTLs have been isolated from normal donors and patients with HER2\(^+\) tumors (4–7). However, lytic activity of separated CTLs has been low because HER2 is a self-antigen attributable to induction of tolerance, and, therefore, HER2-specific T cells display a low avidity T-cell receptor. On this basis, we investigated whether the lytic potential of HER2-specific CTLs could be improved with the help of trastuzumab, an inhibitory antibody against HER2. On binding of anti-HER2 mAb trastuzumab, the receptor is internalized and degraded, subsequently inhibiting HER2-mediated signal transduction and tumor cell growth (8–10). Theoretically, degradation of the internalized HER2 might increase the amount of HER2 protein available for loading into MHC class I molecules, subsequently leading to an enhanced tumor lysis by HER2-specific CTL.

Here we show that anti-HER2 mAb trastuzumab sensitizes HER2\(^+\) tumor cells to lysis by HER2-specific CTLs in vitro. HER2-reactive T-cell clones generated from healthy donors and breast cancer patients lyse class I-matched, HER2-overexpressing tumor cells more efficiently after incubation with trastuzumab. These findings encourage the further development of a combined immunotherapy with trastuzumab and autologous HER2-specific CTLs for patients with HER2-overexpressing tumors.

Materials and Methods

Cells. The HER2\(^+\) ovarian cancer cell line SKOV3 (HLA phenotype: A3, A28, B18, and B35) and the HER2\(^+\) breast cancer cell line SKBR3 (HLA phenotype: A11, B40, and B18) were obtained from American Type Culture Collection (Manassas, VA). The HLA-A2\(^+\) transfectant cell line SKOV3A2 was a gift from M. L. Disis (University of Washington, Seattle, WA). SKOV3A2 express the TIR on the cell surface as determined by fluorescence-activated cell sorting analysis (data not shown). The HLA-A2\(^+\), transporter associated with antigen processing (TAP)-deficient T2 cell line was provided by P. Cresswell (Yale University, New Haven, CT). Tumor cell lines and T2 cells were cultured in RPMI 1640 (Life Technologies, Inc., Paisley, Scotland) supplemented with 10% heat-inactivated FCS, 100 units/ml penicillin, 100 \(\mu\)g/ml streptomycin, and 2 mM glutamine. For down-regulation experiments, confluent SKOV3A2 cells were cultured in the presence of 100 \(\mu\)g/ml anti-HER2 mAb trastuzumab (Herceptin; Roche, Grenzach-Wyhlen, Germany) or 100 \(\mu\)g/ml anti-CD71 mAb (clone 32001A; BD Pharmingen, San Diego, CA) for 2 days.

HER2-specific, class I-restricted CTL clones were generated from different donors by repetitive stimulation with autologous DCs as described recently (6–7). In brief, HLA-A3-restricted CTLs FS-32 and HLA-A2-restricted CTLs FS-D10 and HR-8 were generated from different donors by repetitive stimulation with autologous stem cell-derived DCs (11) retrovirally transduced with HER2. HLA-A2-restricted CTLs NK-1 and IR-1 were established by stimulation with monocyte-derived autologous DCs pulsed with peptides known to be naturally presented with HLA-A2. HER2-specific CTLs NK-1 were generated against HER2\(^{369–377}\), and melan-A-reactive CTLs IR-1 against melan-A\(^{27–35}\). Antigen-specific T-cell lines were cloned by limiting dilution and expanded according to the method described previously by Yee et al. (12).

Western Blot Analysis. Cell lysates were made from SKOV3A2 cells that were cultured in the presence or absence of anti-HER2 mAb trastuzumab or anti-CD71 mAb. Lysates from equal amounts of cells were separated by SDS-PAGE and transferred to nitrocellulose. The HER2 protein was identified using the mAb c-neu-Ab3 recognizing the intracellular domain of HER2 (Oncogene Science, Uniondale, NY) as primary Ab and rabbit antimouse peroxidase-conjugated Fab\(_2\) fragment (Amersham Pharmacia Biotech, Freiburg, Germany) as secondary Ab. The blots were developed using a chemiluminescent reaction (enhanced chemiluminescence; Amersham Pharmacia Biotech).
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Results and Discussion

Drebin et al. (8) have demonstrated that exposure of HER2-transformed cells to inhibitory anti-HER2 antibodies results in the rapid loss of both cell surface and total cellular HER2. This antibody-induced down-regulation of HER2 is a result of accelerated endocytosis and correlates with the tumor-inhibitory potential of the antibody (9). The anti-HER2 mAb trastuzumab was engineered from a murine mAb that inhibits growth of HER2-overexpressing tumors in vitro (10). Western blot analysis of SKOV3tA2 cells expressing the HER2 molecule and the TIR (CD71) was cultured in the absence or presence of either mAb trastuzumab directed against the extracellular domain of HER2 or anti-CD71 mAb. After 2 days, Western blot analysis of SKOV3tA2 cells was performed using mAb c-neu-A3 directed against the intracellular domain of HER2. In comparison to untreated SKOV3tA2 cells (Lane a), trastuzumab-treated SKOV3tA2 cells exhibited reduced total cellular HER2 protein expression (Lane b), whereas SKOV3tA2 cells incubated with anti-CD71 mAb did not down-regulate the HER2 molecule (Lane c).

Cytotoxicity Assay. Cytolytic activity was determined as described previously (6). Briefly, 10⁶ tumor cells were labeled in 100 μl of medium with 0.5 μCi/ml of ⁵¹Cr for 1 h at 37°C. T2 cells were labeled in 100 μl of medium with 200 μCi/ml for 1.5 h at 37°C and then loaded with 10 μg/ml peptide and 3 μg/ml β2-microglobulin (Sigma, Taufkirchen, Germany) for 1 h at room temperature. ³⁵Cr-labeled target cells and graded doses of T cells were given in 200 μl of RPMI supplemented with 10% heat-inactivated human AB serum pooled from healthy donors. Cells were incubated for 4 h at 37°C, 100 μl of supernatant were collected, and radioactivity was measured in a gamma counter. The percentage of specific ⁵¹Cr release was calculated as follows: % specific ⁵¹Cr release = (experimental ⁵¹Cr release – spontaneous ⁵¹Cr release) × 100/(maximum ⁵¹Cr release – spontaneous ⁵¹Cr release). Maximum ⁵¹Cr release was obtained by adding 100 μl of 1% NP40 (Sigma, St. Louis, MO) to 100 μl of labeled target cells. Spontaneous ⁵¹Cr release ranged from 5 to 10% of the total counts incorporated. The data in the figures refer to the mean of two replicates. SDs were <5% of the mean. Similar results were obtained with at least three independent experiments.

To validate the HER2-specific interaction of trastuzumab and CTLs, we next used CTLs with known peptide specificity as effector cells. CTL line NK-1 (6) recognizing HLA-A2-bound peptide HER2b265-277 (4) lysed SKOV3tA2 cells more efficiently after preincubation with trastuzumab (Fig. 3a). HLA-A2-restricted CTL clone IR-1 directed against the melanoma-associated peptide melan-A265-277 (4) did not lyse melan-A-negative SKOV3tA2 cells (Fig. 3b). Culturing SKOV3tA2 cells with trastuzumab did not induce lysis by melan-A-specific CTLs IR-1, indicating that both

Fig. 1. HER2-overexpressing tumor cells down-regulate total cellular HER2 protein expression after ligation with anti-HER2 mAb trastuzumab. Ovarian cancer cell line SKOV3tA2 expressing the HER2 molecule and the TIR (CD71) was cultured in the absence or presence of either mAb trastuzumab directed against the extracellular domain of HER2 or anti-CD71 mAb. After 2 days, Western blot analysis of SKOV3tA2 cells was performed using mAb c-neu-A3 directed against the intracellular domain of HER2. In comparison to untreated SKOV3tA2 cells (Lane a), trastuzumab-treated SKOV3tA2 cells exhibited reduced total cellular HER2 protein expression (Lane b), whereas SKOV3tA2 cells incubated with anti-CD71 mAb did not down-regulate the HER2 molecule (Lane c).

Fig. 2. HER2-specific CTL clones using different restriction elements lyse HER2-overexpressing tumor cells after incubation with anti-HER2 mAb more efficiently. HLA-A2-restricted CTL clone HR-4 (a) and HLA-A3-restricted CTL clone FS-32 (b), both specific for HER2, were generated by repetitive stimulation with autologous DCs retrovirally transduced with HER2. HER2+ SKOV3tA2 cells expressing the class I molecules HLA-A2 and HLA-A3 were lysed by HLA-A2-restricted CTL clone HR-4 and by HLA-A3-restricted CTL clone FS-32 (b). Lysis of SKOV3tA2 cells was enhanced when tumor cells were preincubated with anti-HER2 mAb trastuzumab (b). Lytic activity of CTL clones was not altered when SKOV3tA2 cells were treated with an irrelevant mAb recognizing TIR also expressed by SKOV3tA2 (a), HER2+ SKBR3 (c) being negative for HLA-A2 and HLA-A3 served as negative control.
Fig. 3. Anti-HER2 antibody trastuzumab enhances lysis of HER2\(^{++}\) tumor cells by CTLs recognizing peptide HER2\(^{369-377}\) but not by CTLs specific for peptide melan-A\(^{27-35}\). In a, HLA-A2-restricted CTLs NK-1 recognizes HER2-derived peptide HER2\(^{369-377}\) presented naturally with HLA-A2. T2 cells pulsed with HER2\(^{369-377}\) (\(\bullet\)) served as positive control, and T2 cells pulsed with the irrelevant peptide derived from the influenza A matrix protein\(^{58}\) served as negative control. SKOV3\(^{A}\) cells naturally expressing peptide HER2\(^{369-377}\), together with HLA-A2 were lysed by CTLs NK-1 (\(\bullet\)). Lysis of SKOV3\(^{A}\) cells was augmented after treatment with trastuzumab (\(\bigcirc\)). In b, CTL clone IR-1 lysed T2 cells loaded with melan-A\(^{27-35}\) (\(\blacktriangle\)) but did not lyse T2 cells pulsed with the irrelevant peptide derived from HIV reverse transcriptase (HIV pol\(^{4046-4046}\)). Melan-A-expressing melanoma cell line SKMEL29 (\(\bullet\)) was lysed by CTL clone IR-1. Melan-A-negative SKOV3\(^{A}\) cells were not lysed by CTL IR-1, neither with (\(\blacktriangle\)) nor without (\(\bigcirc\)) preincubation with trastuzumab.

provided evidence that the infusion of antigen-specific T lymphocytes represents an effective modality for the treatment of cancer (12). Up until now, attempts to treat HER2\(^{++}\) tumors by adoptive transfer of HER2-reactive T cells have not been initiated because of the difficulty of generating autologous CTLs directed against the HER2 antigen. We have succeeded in generating and expanding class I-restricted HER2-specific CTL clones (6–7) from patients with HER2-overexpressing breast cancer to numbers that allow adoptive T-cell transfer. The synergistic in vitro antitumor activity of trastuzumab and anti-HER2 CTLs described here encourages the transfer of humoral and cellular immunity to patients who fail to completely eradicate cancer by conventional treatment. Braun et al. (19) observed a correlation between HER2 expression on HER2\(^{++}\) micrometastatic tumor cells in bone marrow and poor prognosis. In this context, the relative resistance of HER2\(^{++}\) micrometastatic cells to adjuvant chemotherapy might be successfully overcome by HER2-targeted immunotherapy that is independent of the proliferative status of the target cells.

Because of the expression of HER2 in some normal tissues, it cannot be ruled out that a combination therapy of trastuzumab and anti-HER2 CTLs might induce autoimmune disease. However, because of low-receptor density on normal cells, the binding of trastuzumab could potentially selectively promote class I-restricted presentation of HER2 peptides on HER2\(^{++}\) tumor cells only. Therefore, the combination of trastuzumab and HER2-specific CTLs with low avidity might lead to tumor rejection without damaging normal tissues in vivo (20). Ongoing studies focus on the toxicity and efficacy of adoptively transferred HER2-specific CTLs with and without trastuzumab in patients with HER2\(^{++}\) breast cancer.

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References


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