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

HER23 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 SKOV3-A2 was a gift from M. L. Disis (University of Washington, Seattle, WA). SKOV3-A2 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 μg/ml streptomycin, and 2 mM glutamine. For down-regulation experiments, confluent SKOV3-A2 cells were cultured in the presence of 100 μg/ml anti-HER2 mAb trastuzumab (Herceptin; Roche, Grenzach-Wyhlen, Germany) or 100 μg/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, HL-60-A3-restricted CTLs FS-32 and HL-60-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. HL-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 HL-A2. HER2-specific CTLs NK-1 were generated against HER2369–377 and melan-A-reactive CTLs IR-1 against melan-A27–35. 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 SKOV3-A2 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 with the help of trastuzumab, an inhibitory antibody against HER2-overexpressing tumor cells more efficiently after treatment with trastuzumab.
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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).

Cytotoxicity Assay. Cytolytic activity was determined as described previously (6). Briefly, 10⁶ tumor cells were labeled in 100 μl of FCS with 100 μCi/ml ⁵¹Cr for 1 h at 37°C. T2 cells were labeled in 100 μl of FCS 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. 3¹Cr-labeled target cells and graded doses of T cells were given in 200 μl of RPMI supplemented with 10% heat-inactivated human AB serum prepared 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 ³¹Cr release was calculated as follows: % specific ³¹Cr release = (experimental ³¹Cr release – spontaneous ³¹Cr release) × 100/(maximum ³¹Cr release – spontaneous ³¹Cr release). Maximum ³¹Cr release was obtained by adding 100 μl of 1% NP40 (Sigma, St. Louis, MO) to 100 μl of labeled target cells. Spontaneous ³¹Cr 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.

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⁺ tumorderived cells via receptor internalization (13). In our experimental in vitro model, antibody trastuzumab, on ligation to its receptor HER2, leads to HER2 down-regulation of total cellular HER2 protein expression in HER2⁺ ovarian cancer cells SKOV3tA2 (Fig. 1). An anti-CD71 mAb, known to induce internalization of the TIR (CD71), was used as an irrelevant antibody control. This anti-CD71 mAb did not influence the HER2 protein level of HER2⁺ ovarian cancer cells coexpressing the TIR.

It has been shown recently by Klapper et al. (10) that tumor-inhibitory antibodies to HER2 enhances ubiquitination of HER2. Protein tagged to ubiquitin is known to be targeted to the proteasome, which cleaves the protein into small peptides. These peptides are then transported into the endoplasmic reticulum, loaded on MHC class I molecules, and transferred to the cell surface for recognizing CTLs. On the basis of these findings, we next examined whether tumor cells that had down-regulated the HER2 receptor on antibody ligation could become sensitized to lysis by HER2-specific CTLs. We cultured the HER2⁺ ovarian cancer cell line SKOV3tA2 in the presence of trastuzumab. The trastuzumab-treated SKOV3tA2 cells were then used as target cells for class I-restricted HER2-reactive CTL clones in a cytotoxicity assay. The HER2-specific CTL clones (6) were derived from different donors by in vitro stimulation with autologous DCs transduced retrovirally with HER2. The HER2-specific CTL clone HR-4, generated from an HLA-A2⁺ patient with HER2⁺ breast cancer, lysed the HER2⁺ SKOV3tA2 cells in an HLA-A2-restricted manner (Fig. 2a). The amount of specific tumor cell lysis correlated with the number of HR-4 CTLs present during the cytotoxicity assay. Pretreatment of SKOV3tA2 cells with trastuzumab resulted in a remarkable increase of tumor cell lysis by CTL clone HR-4. In contrast to nontreated tumor cells, lysis of trastuzumab-treated tumor cells was already detectable at a very low E:T ratio of 1:1. In general, much fewer effector CTLs were needed to lyse equal numbers of antibody-treated tumor cells compared with nontreated tumor cells (e.g., 30% of tumor cell lysis at E:T of 10:1 versus 90:1). Moreover, maximum tumor cell lysis by CTL clone HR-4 was higher (47% versus 26%) and reached at a lower E:T ratio (30:1 versus 90:1), when tumor cells had been incubated with the anti-HER2 antibody. The anti-CD71 mAb capable of inducing TIR internalization did not enhance HR-4-mediated lysis of CD71⁺ SKOV3tA2. Therefore, it can be excluded that enhanced lysis of tumor cells is a general phenomenon observed after receptor internalization.

We next asked whether trastuzumab-treated HER2⁺ tumor cells were also more efficiently lysed by HER2-specific CTLs using a restriction element other than HLA-A2. CTL clone FS-32 recognizing HER2 in context with HLA-A3 was used for lysing HLA-A3⁺ SKOV3tA2, which had been cultured in the presence or absence of anti-HER2 and anti-TIR antibody, respectively (Fig. 2b). Similar to HLA-A2-restricted CTL clone HR-4, the lytic activity of HLA-A3-restricted CTL clone FS-32 was enhanced markedly when tumor cells were preincubated with anti-HER2 mAb trastuzumab. Again, the tumor lysis by CTLs remained unchanged after the incubation of the tumor cells with anti-TIR mAb.

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 HER2⁵⁶⁹–⁷⁷⁷ (4) lysed SKOV3tA2 more efficiently after preincubation with trastuzumab (Fig. 3a). HLA-A2-restricted CTL clone IR-1 directed against the melanoma-associated peptide melan-A₂₇–₃₅ 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. 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 were 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 HER2369–377 but not by CTLs specific for peptide melan-A \(_{27}\). In a, HLA-A2-restricted CTLs NK-1 recognizes HER2-derived peptide HER2369–377 presented naturally with HLA-A2. T2 cells pulsed with HER2369–377 (▲) served as positive control, and T2 cells pulsed with the irrelevant peptide derived from the influenza A matrix protein \(_{286–295}\) served as negative control. SKOV3/A2 cells naturally expressing peptide HER2369–377 together with HLA-A2 were lysed by CTLs NK-1 (●). Lysis of SKOV3/A2 cells was augmented after treatment with trastuzumab (■). In b, CTL clone IR-1 lysed T2 cells loaded with melan-A \(_{27}\) (▼) but did not lyse T2 cells pulsed with the irrelevant peptide derived from HIV reverse transcriptase (HIV poly, 446–465, ▼). Melan-A-expressing melanoma cell line SKMEL29 (●) was lysed by CTL clone IR-1. Melan-A-negative SKOV3/A2 cells were not lysed by CTL IR-1, neither with (●) nor without (■) preincubation with trastuzumab.

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References


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