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A B7.1-Abdomin Protein with Antigen Specificity and Ability to Activate Via the T Cell Costimulatory Pathway

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We describe the construction and characterization of an Ab fusion protein specific for the tumor-associated Ag HER2/neu linked to sequences encoding the extracellular domain of the B7.1 T cell costimulatory ligand. The Ab domain of the fusion molecule will specifically target HER2/neu-expressing tumor cells, while the B7.1 domain is designed to activate a specific immune response. We show that the B7.1 fusion Ab retained ability to selectively bind to the HER2/neu Ag and to the CTLA4/CD28 counter-receptors for B7.1. Specific T cell activation was observed when the B7.1 Ab fusion protein was bound to HER2/neu-expressing cells. The use of the B7.1 Ab fusion protein may overcome limitations of gene transfer and/or standard Ab therapy and represents a novel approach to the eradication of minimal residual disease. The Journal of Immunology, 1998, 160: 3419–3426.

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Materials and Methods

Cell lines and reagents

CHO, EL4, SKBR3, Sp2/0, and P3X63-Ag.653 cells were available in the laboratory or obtained from American Type Cell Collection (Rockville, MD). EL4, Sp2/0, and P3X63Ag8.563 cells were cultured in Iscove’s medium supplemented with 5% FBS and L-glutamine, penicillin, and streptomycin (GPS). SKBR3 cells were grown in RPMI 1640 medium containing 10% FBS and GPS. CHO cells were maintained in DMEM supplemented with 10% FBS and GPS. CHO/CD28 and CHO/B7 cells as well as the CD28Ig and B7Ig soluble proteins were provided by Dr. P. Linsley (Bristol-Myers-Squibb Pharmaceutical Research Institute, Seattle, WA). CHO/CD28 and CHO/B7 cells were grown in the same medium as CHO cells supplemented with 0.2 mM proline and 1 μM methotrexate. CHO cells transfected with the HER2/neu CDNA were maintained under selection with 0.5 mg/ml of Genetin (Life Technologies, Gaithersburg, MD). Soluble CTLA4Ig was purified from a hybridoma obtained from Dr. J. Allison (University of California, Berkeley, CA) using standard protein A column purification methods.

Expression vectors

HER2/neu retrovector and gene delivery. The plasmid encoding the human HER2/neu cDNA (clone 0483 generously provided by Genentech, Inc., San Francisco, CA) was digested with HindIII, filled in using Klenow polymerase, digested with XhoI, and cloned into the XhoI and filled-in BamHI sites of the retroviral vector LXSN (16). The resulting plasmid was transfected into the PA317 packaging cell line using Lipofectin reagent (Life Technologies) and cells selected in 0.5 mg/ml Genetin. Culture supernatant from the vector-producing PA317 cells was harvested, filtered through 0.45-μm pore size filters, and used to transduce CHO cells to derive CHO/Her2 cells.

Abbreviations used in this paper: GPS, L-glutamine, penicillin, and streptomycin; Ser, serine; Gly, glycine; PE, phycoerythrin; CHO, Chinese hamster ovary.

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Anti-HER2/neu κ light chain expression vector. The light chain V domain of the humanized humAb4DS-8 Ab was amplified from the plasmid pAK19 (17) (provided by Dr. P. Carter, Genentech) and fused to the 3′-end of human κ leader sequence by overlapping PCR. The primers used in the first cycle of amplification are: primer a, 5′-GGGGATATCCACCATGG(T/G)ATG(C/G)ACTGG-3′ and b, 5′-GACTCCACAGGTGTCCACCTCGGAGTGCGTGACCAG-3′ for the leader sequence. The final 500-bp PCR product encoding the leader sequences as template DNA; and c, 5′-CTCCACAGGTGTCCACCTCGGAGTGCGTGACCAG-3′ and d, 5′-GCTTGTGAGTCTTTGGGATATCCACCATGTCCACCTCGGAGTGCGTGACCAG-3′ for the Vκ sequences using pAK19 as template DNA. The resultant PCR products were mixed and used as template for the amplification with primers a and d. The final PCR product of 470 bp was digested with EcoRV and SalI and cloned into the human κ light chain expression vector previously described (18).

Anti-HER-2/neu heavy chain expression vector. The strategy used to clone the heavy chain variable domain (VH) from pAK19 is similar to the Vκ cloning strategy. The primers used for amplification are: primer a, 5′-GGGGATATCCACCATGG(T/G)ATG(C/G)ACTGG-3′ and b, 5′-GACTCCACAGGTGTCCACCTCGGAGTGCGTGACCAG-3′, c, 5′-CTCCACAGGTGTCCACCTCGGAGTGCGTGACCAG-3′ and d, 5′-GCTTGTGAGTCTTTGGGATATCCACCATGTCCACCTCGGAGTGCGTGACCAG-3′, and e, 5′-GATCTCCACAGGTGTCCACCTCGGAGTGCGTGACCAG-3′. The final PCR product of 470 bp was digested with EcoRV and SalI and cloned into the human IgG3 mammalian expression vector previously described (18).

Anti-HER-2/neu B7.her2.IgG3 fusion heavy chain expression vector. The extracellular domain of the human B7.1 including the leader sequences was amplified using primers 5′-GGCGGCGGATCCGAGGTTCAGCTGGTG-3′ and 3′-GG(A/G)ATG(C/G)AGCTG(T/G)GT(C/A)AT(G/C)CTCTT-3′. The Vκ domain of the humanized humAb4DS-8 Ab was amplified by PCR from the plasmid pAK19 using primers 5′-GGCGGCGGATCCGAGGTTCAGCTGGTG-3′ and 3′-GG(A/G)ATG(C/G)AGCTG(T/G)GT(C/A)AT(G/C)CTCTT-3′. The resulting PCR products were mixed and used as template for amplification with primers a and d. The final PCR product of 470 bp was digested with EcoRV and SalI and cloned into the human IgG3 mammalian expression vector previously described (18).

Recombinant Ab expression, immunoprecipitation, and purification Purified recombinant anti-HER2/neu Ab alone is referred to in the manuscript as her2.IgG3, and the anti-HER2/neu Ab fused to B7.1 is referred to as B7.her2.IgG3. Transfection, expression, and purification of the recombinant Abs were performed as described previously (19). Briefly, nonsecreting Sp2/0 or P3X63-Ag.653 myeloma cells were transfected with 10 μg of each of the anti-HER2/neu light chain and heavy chain expression vectors by electroporation. Transfected cells were plated at 10,000 cells/well in 96-well tissue culture plates. The next day, selection in 0.5 μg/ml Neomycin (Sigma) was initiated and maintained for 10 to 14 days. Wells were screened for Ab secretion by human IgG-specific ELISA as previously described, and positive wells were expanded. To determine the size of the secreted recombinant Abs, supernatants from cells grown overnight in medium containing [35S]methionine were immunoprecipitated with goat anti-human IgG (Zymed Laboratories, Inc., San Francisco, CA) and staphylococcal protein A (IgGorb, The Enzyme Center, Malden, MA). Precipitated Abs were analyzed on SDS-polyacrylamide gels in either the presence or the absence of reducing agents. For purification of her2.IgG3 and B7.her2.IgG3 Abs, Ab secreting Sp2/0 clones were expanded in roller bottles in Hydridoma Serum-Free Medium (Life Technologies), and 2 to 4 l of cell-free medium was collected. Culture supernatants were passed through a GammaBind protein G column (Pharmacia Biotech, Inc., Piscataway, NJ), and the column was washed with 10 ml of PBS. The protein was successively eluted with a total of 10 ml of 0.1 M glycine at pH 4.0, 2.5, and 2.0, and the eluate was neutralized immediately with 2 M Tris-HCl, pH 8.0. The eluted fractions were dialyzed and concentrated using Centricon filters with a molecular mass cut-off of 30,000 Da (Amicon, Inc., Beverly, MA).

Flow cytometry studies Cells were detached by treatment with 0.5 mM EDTA, washed, and incubated with recombinant her2.IgG3 or B7.her2.IgG3 antibodies for 1 to 2 h at 4°C, then washed and stained with FITC-conjugated anti-human IgG (Sigma) or PE-conjugated anti-human B7.1 (Becton Dickinson, San Jose, CA) and analyzed by flow cytometry.

Affinity analysis The affinity of her2.IgG3 fusion protein for the Ag was compared with that of the parental her2.IgG3 Ab using the 1Asys Optical Biosensor from Fisons Applied Sensor Technology (Paramus, NJ). Soluble HER2/neu Ag (ECD, provided by Genentech) was immobilized on a sensitized microcuvette according to the manufacturer’s instructions. Her2.IgG3 or B7.her2.IgG3, at different concentrations in PBS with 0.05% Tween-20, was added to the cuvette, and association and dissociation were measured. Rate constants were calculated using the FASTfit software (supplied with the 1Asys System) as previously described (20).

T cell proliferation assays Human PBMC were isolated from normal donor blood using standard Ficoll-Hypaque density centrifugation. Human T cell enrichment columns (R&D Systems, Minneapolis, MN) were used for T cell purification according to the manufacturer’s instructions. Purified T cells were plated in flat-bottom 96-well tissue culture plates at 1 × 105 cells/well in RPMI supplemented with 5% FBS. Irradiated (5000 rad) CHO, CHO/Her2 or CHO/B7 cells were added at 2 × 104 cells/well in the presence of 0, 1, 5, or 10 μg/ml recombinant her2.IgG3 or B7/her2.IgG3 and 10 ng/ml PMA (Sigma). Plates were incubated at 37°C for 3 days, pulsed with 0.6 μCi/ml of [3H]thymidine for 16 to 18 h, and harvested, and [3H]thymidine incorporation was measured.

Results Design and expression of the recombinant Abs The expression vectors for the human IgG3 heavy and κ light chains were previously described (18). The variable domains of the anti-HER-2/neu Ab were amplified by PCR from the plasmid pAK19 (provided by P. Carter, Genentech) (17) and cloned into the corresponding heavy or light chain expression vectors to derive her2.IgG3. To construct a fusion Ab between her2.IgG3 and B7.1 (referred to as B7.her2.IgG3), the extracellular domain of human B7.1 was cloned at the 5′-end of the heavy chain variable region of her2.IgG3 (Fig. 1A). A flexible (Ser-Gly)4 linker was provided at the fusion site of the recombinant fusion protein to facilitate correct folding of both Ab and B7.1 domains. We elected to express B7.1 at the amino terminus of the heavy chain because B7.1 fused to the carboxyl terminus of the Ck3 domain showed decreased affinity for CD28 (data not shown). These results are consistent with a critical role for the amino terminus of B7.1 in mediating its biologic activity (21). The light chain and either the her2.IgG3 or B7.her2.IgG3 heavy chain expression vectors were cotransfected into Sp2/0 myeloma cells, and stable transfectants secreting soluble proteins were identified by ELISA.

To determine the molecular mass and assembly of the transfected proteins, cells were grown overnight in [35S]methionine, and the secreted proteins were immunoprecipitated and analyzed by SDS-PAGE. In the absence of reducing agents, her2.IgG3 migrates with an apparent molecular mass of 170 kDa, while B7.her2.IgG3 is about 250 kDa (Fig. 1, lanes 1 and 2, respectively). Following treatment with 2-ME, light chains of 25 kDa were seen for both proteins, while her2.IgG3 had a heavy chain of approximately 60 kDa, and B7.her2.IgG3 had a heavy chain of approximately 100 kDa (Fig. 1B, lanes 3 and 4, respectively). Therefore, proteins of the expected molecular mass were produced, and the fusion of the extracellular domain of B7.1 to the her2.IgG3 heavy chain did not alter the secretion of the fully assembled H2 L2 form of the Ab.

Ag binding We tested the ability of recombinant her2.IgG3 and B7.her2.IgG3 to bind to the HER2/neu antigenic target by flow cytometry (Fig. 2). CHO cells stably expressing the HER2/neu Ag (CHO/Her2) derived by retroviral-mediated gene transfer and nontransduced CHO cells were cotransfected with either her2.IgG3 or B7/her2.IgG3. Binding was assayed by staining with either FITC-conjugated anti-
human IgG or PE-conjugated anti-human B7.1 Abs followed by flow cytometry. Both her2.IgG3 (Fig. 2, A and B) and B7.her2.IgG3 (Fig. 2, D and E) bound specifically to CHO/Her2 and not to parental CHO cells. Therefore, fusion of the extracellular domain of B7.1 to a complete her2.IgG3 Ab resulted in a fusion Ab capable of specifically recognizing the HER2/neu Ag through the Ab domain. CHO/Her2 cells incubated with B7.her2.IgG3 also stained positively with anti-human B7.1, indicating that binding of B7.her2.IgG3 to the Ag through its Ab domain did not interfere with Ab recognition of the B7.1 fusion domain (Fig. 2F).

The affinities of the her2.IgG3 and B7.her2.IgG3 Abs for the HER2/neu Ag were compared using the IAsys biosensor (Fig. 3). Her2.IgG3 or B7.her2.IgG3, at $1 \times 10^{-7}$ M, was added to a cuvette with soluble HER2/neu Ag ECD immobilized on its surface, and the association and dissociation were measured as the samples were added and washed from the cuvette. The calculated affinity of $1.7 \times 10^{-7}$ M for B7.her2.IgG3 was decreased about 2.5-fold compared with the affinity of $7 \times 10^{-8}$ M obtained for the parental her2.IgG3. The modest decrease in affinity primarily reflected a reduction in the dissociation constant of B7.her2.IgG3.

**B7.1 binding studies**

The ability of the B7.1 domain in the B7.her2.IgG3 fusion protein to bind to its receptors CTLA4 and CD28 was studied by two different methods. Soluble CTLA4lg and CD28Ig immobilized on nitrocellulose membrane were incubated with either her2.IgG3 or B7.her2.IgG3 (Fig. 4A). We observed strong binding of B7.her2.IgG3 to CTLA4lg, but no binding of her2.IgG3. B7.her2.IgG3 also bound CD28Ig, although with a lesser affinity than to CTLA4lg. This was expected, since the reported affinity of B7.1 for CTLA4 is 20-fold higher than that for CD28 (22). In another experiment, we used CHO cells stably expressing CD28 to detect B7.her2.IgG3 binding (Fig. 4B). Parental CHO or CHO/
CD28 cells were incubated with either B7Ig (a gift from Dr. P. Linsley) or B7.her2.IgG3 and washed, and binding was detected by staining with FITC-conjugated anti-human IgG followed by flow cytometry. Specific binding of B7.her2.IgG3 and B7Ig to CD28 present on CHO-CD28 cells, but not to control CHO cells, was observed.

Stability of the anti-HER2/neu recombinant Abs on the cell surface

Since recruitment and activation of tumor-specific T cells would depend on the presence of B7.1 on the tumor cell surface, we characterized the stability of B7.her2.IgG3 bound to the HER2/neu Ag expressed on the cell membrane. SKBR3 cells from a human breast cancer cell line known to express high levels of HER2/neu were incubated with 10 μg/ml of either her2.IgG3 or B7.her2.IgG3 at 4°C for 2 h. The cells were washed and stained with either FITC-conjugated anti-human IgG (A, B, D, E) or PE-conjugated anti-human B7.1 (C and F) at 4°C for 30 min. The cells were then analyzed by flow cytometry. This experiment was repeated six times, and similar results were observed each time.

CD28 cells were incubated with either B7Ig (a gift from Dr. P. Linsley) or B7.her2.IgG3 and washed, and binding was detected by staining with FITC-conjugated anti-human IgG followed by flow cytometry. Specific binding of B7.her2.IgG3 and B7Ig to CD28 present on CHO-CD28 cells, but not to control CHO cells, was observed.

FIGURE 2. Flow cytometry to detect binding of her2.IgG3 or B7.her2.IgG3 to cell-surface-expressed HER2/neu Ag. Parental CHO (A and D) or CHO/Her2 (B, C, E, and F) cells were incubated with 10 μg/ml of either her2.IgG3 (A–C) or B7.her2.IgG3 (D–F) at 4°C for 2 h. The cells were washed and stained with either FITC-conjugated anti-human IgG (A, B, D, E) or PE-conjugated anti-human B7.1 (C and F) at 4°C for 30 min. The cells were then analyzed by flow cytometry. This experiment was repeated six times, and similar results were observed each time.

FIGURE 3. Affinity of her2.IgG3 and B7.her2.IgG3 for HER2/neu determined using the IAssys biosensor. Binding of her2.IgG3 or B7.her2.IgG3 to a HER2/neu extracellular domain-coated microcuvette was assayed as described in Materials and Methods, and $k_s$ and $k_r$ were calculated using the Fastfit program. The affinity constant $K_d$ was calculated as $k_s/k_r$.

FIGURE 4. Binding of B7.1 to its counter-receptors CD28 and CTLA4 determined by slot blot (A) or flow cytometric (B) cells. A, One hundred or twenty nanograms of CTLA4Ig or CD28Ig immobilized on a nitrocellulose membrane was incubated with either purified her2.IgG3 or B7.her2.IgG3 followed by alkaline phosphatase-conjugated anti-human κ. The blots were then developed with 5-bromo-4-chloro-3-indolyl-phosphate/nitro blue tetrazolium chloride substrate. B, Parental CHO (a and c) or CHO/CD28 cells (b and d) were incubated with soluble human B7.1 in the form of B7Ig (a and b) or with B7.her2.IgG3 fusion protein (c and d). The cells were then washed, incubated with FITC-labeled anti-human IgG, and analyzed by flow cytometry.

Proliferation assays

To test for the functional ability of the B7.her2.IgG3 molecule to signal via CD28, we performed a syngeneic T cell proliferation assay using human peripheral blood T cells (Fig. 6). CHO/Her2 or control CHO cells were irradiated and incubated in the presence or the absence of either her2.IgG3 or B7/her2.IgG3 and peripheral blood-enriched T cells. PMA at 10 ng/ml was added to the cultures to provide signal 1, which was necessary for proliferation. Addition of B7/her2.IgG3 to CHO/Her2 cells resulted in a dose-dependent increase in T cell proliferation as assayed by [3H]thymidine incorporation.
with her2.IgG3 (a) of Ag-expressing breast cancer cells. SKBR3 cells were incubated at 4°C surface via the HER2/neu ligand in preference to B7.2, as Gajewski et al. and other investigators have suggested that B7.1-transduced tumors more successfully induce CTL activity and protect against parental tumor challenge more effectively than tumors transduced with B7.2 (24–27). Although conflicting results with respect to Th1 vs Th2 differentiation have been reported using B7.1 and B7.2, results from several experimental systems suggest that B7.1 costimulation tends to favor differentiation along the Th1 pathway (1, 28–30). Therefore, we chose to link B7.1, rather than B7.2, to an antitumor Ab in an effort to preferentially stimulate a Th1-mediated immune response.

Our results indicate that B7.1 can be effectively linked to the amino terminus of the heavy chain of an anti-HER2/neu Ab, with retention of both Ab specificity and the B7.1 interaction with CD28. Binding to HER2/neu was demonstrated by flow cytometry as well as IAys biosensor studies, albeit at a lower affinity than that observed for the control her2.IgG3. Possible reasons for the observed decrease in affinity could be steric hindrance between the anti-HER2/neu variable and the B7.1 domains or a change in the kinetics of Ag binding due to the increased size of B7.her2.IgG3. Similarly, specificity of B7.1 for both CTLA4 and CD28 was demonstrated by the ability of B7.her2.IgG3 to bind soluble CTLA4Ig and CD28Ig as well as CD28 expressed on the surface of target cells. Of note, in preliminary attempts to derive a fusion Ab, we constructed an anti-dansyl Ab fusion in which the B7.1 coding domain remains intact in B7.her2.IgG3, binding to Fc receptors might disrupt the B7.1/CD28 interaction. This may be due to masking of the amino terminal sequences of B7.1. Since the Fc region of IgG3 would be expected to provide greater flexibility in folding to accommodate the presence of B7.1 in the fusion Ab, IgG3 also exhibits Fc-mediated functions, such as complement activation and Fcy binding (23). We chose the B7.1 costimulatory ligand for its ability of B7.her2.IgG3 to bind soluble CTLA4Ig, suggesting that fusion through the B7.1 amino terminus may disrupt the B7.1/CD28 interaction. This may be due to masking of the amino terminal sequences of B7.1, which are known to be in close proximity to the CD28/CTLA4 binding site (21). Whether fusion via a flexible linker will restore binding of B7.1 to CD28 is currently under investigation. At present, however, we would favor fusion of B7.1 sequences to the amino-terminal heavy chain sequences for suitable B7.1/CD28 interaction. A similar requirement for fusion at the amino terminus of the Ab to maintain activity was observed for nerve growth factor (31). Since the Fc domain remains intact in B7.her2.IgG3, binding to Fc receptors may induce Ag-dependent cellular cytotoxicity or otherwise affect B7.1 function. If this is a problem, further manipulation of the constant region could be performed to eliminate FcR binding sites.

We also sought to address whether anti-HER2/neu Abs would remain on the surface of Ag-presenting breast cancer cells. Our results indicated that approximately 40% of surface-bound B7.her2.IgG3 was detectable by flow cytometry for up to 24 h following initial incubation with human SKBR3 breast cancer cells. This suggests that stable presentation of the B7.1 costimulatory ligand on the tumor cell surface may be feasible, and that loss of presentation due to internalization or rapid antigenic shedding via HER2/neu binding may not be a significant problem (32).

The management of minimal residual disease is a central problem in breast cancer and other solid tumors. Despite the use of...
FIGURE 6. In vitro T cell proliferation assay. Peripheral blood T cells isolated from blood of normal donors A and B were plated in 96-well plates in the presence of irradiated CHO or CHO/Her2 cells, PMA (10 ng/ml), and increasing concentrations of either her2.IgG3 or B7.her2.IgG3. The cocultures were incubated at 37°C for 3 days and labeled with [3 H]thymidine for the final 16 to 18 h. A, Proliferation was measured by harvesting the cells onto glass filters and assessing radioactivity by liquid scintillation counting. The results shown represent the average of triplicate cultures, and error bars denote the SE. Results from two separate experiments using two separate donors, donor A and donor B, are shown. The experiment was repeated five times using a total of three different T cell donors with similar results. B, Photographs are shown following 3 days of human T cell incubation in the presence of a) CHO/Her2 cells and 10 μg/ml her2.IgG3, b) CHO/Her2 and 10 μg/ml B7.her2.IgG3, c) CHO cells and 10 μg/ml B7.her2.IgG3, d) CHO/Her2 in the absence of Ab, or e) CHO/B7 cells stably expressing human B7.1 by gene transfer.
increased dose intensity of chemotherapy or autologous bone marrow transplantation, relapse remains a critical problem (33–36). Chemotherapeutic strategies are necessarily limited by various toxicities. Additional modalities that can achieve further cytoreduction are needed. Although various clinical trials of mAbs, Ab-based conjugates, and/or radioantibodies have been performed, the results of these trials have highlighted obstacles to successful Ab-based therapy of human malignancy. Abs generally are not directly cytotoxic due to poor fixation of complement and/or inadequate activation of Ab-dependent cytotoxicity. Effective use of Abs for delivering cytotoxic agents (e.g., conjugates such as Ab-rin, or radiolabeled Ab strategies) requires delivery to a majority of, if not all, tumor cells (37). An alternative approach is to elicit an active systemic immune response against tumor cells. Delivery of cytokines has been shown to induce an antitumor T cell response. Although gene transfer has most commonly been used to achieve increased cytokine levels at the site of the tumor, recent studies performed using an Ab-IL-2 fusion protein suggest that Abs can be used for delivering cytokines to tumors. The Ab-cytokine fusion protein retains both Ab specificity and cytokine activity and appears to be more effective than either used alone or in combination, but not covalently linked (38–43). However, fusion of B7.1 rather than cytokine should result in activation of T cells with TCRs that specifically recognize tumor determinants rather than the nonspecific activation expected of a fused cytokine.

In assays for T cell costimulation in vitro, we show that effective stimulation of human T cells was achieved only if B7.her2.IgG3 was bound to a HER2/neu target, and limited or no stimulation was observed using target cells that did not express HER2/neu Ag. This suggests that the B7.her2.IgG3 fusion protein in soluble form may not be able to effectively provide a costimulatory signal to preactivated T cells, and that the anti-HER2/neu Ab domain in the B7.her2.IgG3 fusion protein provided specificity for the T cell costimulation. This property of B7.her2.IgG3 would allow enhanced specificity of the immune response. Similar results have been reported using fusion of the B7.2 costimulatory ligand to a single chain Ab (44). However, a single chain Ab produced in yeast cells may have considerably different glycosylation and antigenicity as well as different pharmacokinetics in vivo compared with the humanized B7.her2.IgG3 we have produced. The relative specificity and type of response achieved with B7.her2.IgG3 fusion compared with the B7.2 single chain fusion remain to be determined. It also remains to be determined whether the specificity and response achieved with B7.1 fusions will differ from those observed using bispecific antitumor/anti-CD28 Ab (45). However, genetically engineered Ab fusion proteins should present fewer problems in manufacture and purification than the described bispecific Abs, which are difficult to purify to homogeneity.

In summary, we have produced an anti-HER2/neu IgG fusion protein encoding the extracellular domain of the B7.1 costimulatory ligand. This protein retains targeting specificity via the HER2/neu Ag as well as ability to deliver a T cell costimulatory signal. The strategy offers several theoretical advantages. While expression of HER2/neu may be heterogeneous, targeting via HER2/neu may activate T cells with specificity against other unidentified tumor-associated Ags, resulting in destruction of both HER2/neu-positive and nonexpressing cells. Therefore, the Ab fusion protein may allow targeting of micrometastatic disease with relative specificity and would not itself have to bind to all tumor cells to elicit an effective response. The data presented suggest that tumor-specific Abs fused with costimulatory ligands may be a useful method for delivering a costimulatory signal for the purpose of cancer immunotherapy.


