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Antitumor Response Elicited by a Superantigen-Transmembrane Sequence Fusion Protein Anchored onto Tumor Cells

Jennifer L. Wahlsten, Charles D. Mills, and S. Ramakrishnan

Superantigens stimulate T cells bearing certain TCR β-chain variable regions when bound to MHC II molecules. We investigated whether the superantigen toxic shock syndrome toxin-1 (TSST1) could induce an antitumor immune response when anchored onto MHC II-negative tumor cells. Our approach was to facilitate association of TSST1 with cell membranes by fusing its coding region to the transmembrane region (TM) sequence of the proto-oncogene c-erb-B-2. TSST1-TM was expressed in bacteria with an N-terminal histidine tag and purified using nickel-agarose affinity chromatography. Purified TSST1-TM added to cultures of several different MHC II-negative tumor cells spontaneously associated with cell membranes, as detected by flow cytometry. Because superantigens can direct cell-mediated cytotoxicity against MHC II-positive cells, a TM fusion protein lacking the TSST1 MHC II binding domain (TSST88–194-TM) was also constructed. Tumor cells precoated with TSST1-TM or TSST88–194-TM stimulated proliferation of peripheral blood lymphocytes in vitro whereas uncoated tumor cells did not. Mice preimmunized with TSST1-TM- or TSST88–194-TM-coated tumor cells mounted a systemic response that resulted in significant antitumor immunity as measured by regression of a parental tumor challenge. TSST1-TM and TSST88–194-TM fusion proteins represent a useful new strategy for attaching superantigens or potentially other proteins onto tumor cell surfaces without genetic manipulation. The Journal of Immunology, 1998, 161: 6761–6767.

Tumor cells often escape protective immune responses because they lack or down-regulate stimulatory surface Ags (1). Introduction of immunostimulatory Ags such as MHC I, MHC II, or B7-1 onto the surface of tumor cells can induce antitumor immunity as demonstrated by rejection of parental tumor in vivo (2). Typically, these approaches involve transfection of the relevant genes. However, transfection is time consuming, and primary tumor cells often do not grow well in vitro. Therefore, the practical application of this strategy to human tumors may be limited.

Alternatively, surface Ags can be introduced onto tumor cells without transfection through fusion with a glycosyl-phosphatidylinositol (GPI)4 signal sequence (3). Purified, recombinant GPI-linked fusion proteins incubated with tumor cells can passively re-anchor onto extracellular membranes. In vitro studies applying this approach demonstrated that GPI-anchored, Ag-presenting MHC I molecules effectively promoted T cell-mediated cytotoxicity (4), and GPI-anchored B7-1 (CD80) costimulated lymphocyte proliferation (5).

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6 Address correspondence and reprint requests to Dr. S. Ramakrishnan, Department of Pharmacology, University of Minnesota, 3-249 Millard Hall, 435 Delaware Street SE, Minneapolis, MN 55455.
7 Abbreviations used in this paper: GPI, glycosyl-phosphatidylinositol; TSST1, toxic shock syndrome toxin-1; H, histidine tag; TM, transmembrane; T84, TSST1 residues 88–194; LLC, Lewis lung carcinoma; IPTG, isopropyl β-D-thiogalactopyranoside; Mit C, mitomycin C; SEA, Staphylococcal enterotoxin A.
hydrophobicity will change a translocating sequence into a stop-anchor sequence (14, 15), we hypothesized that fusing TSST1 to a hydrophobic TM sequence would permit it to passively anchor onto cell membranes of living cells. Here we show that a recombinant TSST1-transmembrane region chimera (HTSTS1-TM) spontaneously associates with intact cells.

Superantigen-based antitumor strategies may offer therapeutic promise. However, because superantigens preferentially direct cytotoxicity against MHC II-positive cells (16–18), in vivo administration of intact superantigens in sufficiently therapeutic amounts risks unwanted cytotoxicity against normal cells. Therefore, successful employment of superantigens in tumor immunotherapy will likely require compromising their MHC II binding capabilities. Crystallographic studies have demonstrated that residues within the TSST1 N-terminal domain directly interact with MHC II molecules (19), and mutation analyses of TSST1 have localized residues critical for its superantigenic activity to the C-terminal domain (20–22). When expressed as a recombinant protein, the TSST1 C-terminal residues 88–194 do not bind to MHC II molecules yet retain superantigenic activity (23). Therefore, a TM fusion protein having only the TSST1 C-terminal residues 88–194 was also constructed (HTSTS88–194-TM or HT84-TM). Here we show that cell-anchored HTSTS1-TM and HT84-TM exhibit potent biological activity in vitro and in vivo, as measured by lymphocyte proliferation and tumor immunization, respectively.

Passive anchoring of superantigen and potentially other TM fusion proteins offers a novel strategy for addition of immunostimulatory molecules onto tumor cells.

Materials and Methods

Reagents

The prokaryotic expression vector pET17bH, with an N-terminal histidine sequence, has been described (24). A TSST1 subclone was provided by Dr. P. M. Schlievert (University of Minnesota, Minneapolis). Enzymes for molecular cloning were purchased from New England Biolabs (Beverly, MA). Oligonucleotides were synthesized by Integrated DNA Technologies (Coralville, IA). FITC-labeled goat-anti-rabbit IgG Abs were obtained from Sigma (St. Louis, MO). Murine cell lines EL4 and Lewis lung carcinoma (LLC) (C57BL6, H-2b) and the plasmid encoding c-erb-B-2 were purchased from the American Type Culture Collection (Manassas, VA). Raji MHC II-negative mutant RJ2.2.5 was obtained from Dr. J. M. Boss (Emory University, Atlanta, GA) with permission of Dr. R. Accolla (Advanced Biotechnology Center, Genova, Italy). The murine P815 mastocytoma (DBA/2, H-2d) has been described (25–27). Female C57BL6 × DBA/2 (B6D2F1, H-2{sup}b{sup}) 8-wk-old mice were purchased from Taconic Farms (Germantown, NY) and housed in the University of Minnesota Animal Resources facility. The P815 tumor grows progressively and has been shown to generate a tumor-specific response in B6D2F1 mice as well as in the parental strain (25–27).

Construction and subcloning of the TSST1-TM and T84-TM fusion sequences into pET 17bH

The coding sequences for TSST1 or TSST88–194 were fused to the TM-encoding sequence of c-erb-B-2 using splice overlap extension PCR (28) and the following primers, with restriction sites in bold and an underlined stop codon: primer 1, 5'-CCCAATAGTCCTACAACAGATAATAAAGGAT-3'; primer 2, 5'-TCTCTGTGCGCAGACTTATTTATATTGTGC-3'; primer 3, 5'-GGACAGATTTAATCTTAGTGGCGAGACAGA-3'; primer 4, 5'-GGGGATGTTTACGTTGACTTGGGAGAGA-3'; primer 5, 5'-CCCAATAGTCCTACAACAGATAATAAAGGAT-3'.

The TSST1 coding sequences were amplified from MNT subclone 6101 (29) using primers 1 and 2 or 5 and 2. The DNA sequence corresponding to amino acid residues 644–687 of c-erb-B-2, inclusive of the TM region, was amplified from plasmid clone pCER204 (30) using primers 3 and 4. The amplified fragments corresponding to TSST1 (610 bp), T84 (320 bp), and c-erb-B-2 (160 bp) were purified and used as templates in subsequent PCRs. The TSST1-TM fusion sequences were PCR-amplified from the TSST1 or T84 and c-erb-B-2 fragments using primers 1 and 4 or 5 and 4, respectively. The amplified fragments (740 bp and 450 bp) were digested with NdeI and XhoI and ligated into predigested pET 17bH. Positive clones were confirmed through NdeI/XhoI redigestion and dsDNA sequencing before transforming into host strain BL21(DE3) pLysS for expression.

Expression of HTSTS1-TM and HT84-TM in E. coli

Expression of HTSTS1-TM and HT84-TM in E. coli was adapted from a described protocol (31, 32). Overnight cultures of transformed BL21(DE3) pLysS cells were inoculated 1:40 into 1 liter of M9-ZB medium (33) supplemented with 2% glucose, 100 μg/ml ampicillin, and 34 μg/ml chloramphenicol. Cultures were grown to an OD600 of 0.8–1.0 with continuous shaking at 37°C before transcription was induced with isopropyl-β-D-thiogalactopyranoside (IPTG, 0.4 mM). Cells were harvested after 2-h induction, the cell pellet was resuspended in 80 ml sonication buffer (300 mM NaCl/50 mM sodium phosphate, pH 8.1% Triton X-100), and was frozen at −80°C.

Purification of HTSTS1-TM and HT84-TM

Proteins expressed from pET 17bH have 10 sequential histidine residues at the amino terminus, allowing purification over nickel-agarose. The frozen cell lysate was thawed, sonicated and clarified by centrifugation at 28,000 × g for 30 min. The clarified lysate was diluted with an equal volume of sonication buffer and loaded batchwise onto nickel agarose over 4 h at 4°C. The resin was rinsed with sonication buffer, placed in a column support, and rinsed with wash buffer (300 mM NaCl/50 mM sodium phosphate, pH 8.1% glycerol). Non-specitically bound host proteins eluted in wash buffer containing 100 mM imidazole. HTSTS1-TM and HT84-TM bound to the resin was eluted in wash buffer containing 300 mM imidazole and dialysed into PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM NaHPO4, 1.4 mM KH2PO4). Purified, dialysed protein was concentrated, aliquoted, and stored at −80°C. Protein concentrations were determined using BCA assay reagents (34). Relative protein purity was determined by densitometric measurement of Coomassie brilliant blue R-250-stained 12% SDS-polyacrylamide gels.

Association of HTSTS1-TM with MHC II-negative tumor cells

Tumor cells were resuspended to 0.5–1.0 × 10{sup}6{sup} cells/ml in medium containing 2.5% FCS. Purified HTSTS1-TM was added to a final concentration of 0.33–1.67 μM and incubated for 4 h at 37°C. Cells were rinsed and analyzed for the presence of HTSTS1 on their surface by flow cytometry. In the negative control, cells not exposed to HTSTS1-TM were incubated with primary and secondary Abs. Anchored HTSTS1-TM was detected with purified IgG from rabbit serum against TSST1 residues 88–194 (αTS84), added at a 1:300 dilution with 3% normal goat serum. Bound αTS84 was detected with FITC goat anti-rabbit IgG added at a 1:200 dilution. Cells were gated for viability using propidium iodide and 2–10 × 10{sup}4{sup} events were measured by fluorescence by flow cytometry.

The presence of HTSTS1-TM on the human ovarian carcinoma cell line, MA 148 (35), was also visualized by in situ immunohistochemistry. MA 148 cells were seeded at low density onto glass coverslips and grown for 24–48 h. The cells were then incubated for 4 h at 37°C in medium containing 1 μM HTSTS1-TM and 2.5% serum. Cells were rinsed with PBS, fixed for 10 min at room temperature (4% paraformaldehyde−2% sucrose in PBS). Detection of HTSTS1-TM was done using the Abs described above. Coverslips were mounted onto glass slides. Images were captured with a ×10 neofluor objective on a Nikon Diaphot 300 inverted microscope connected to a Photometrics PXL cooled CCD camera (Tucson, AZ) using the computer program IPlab Spectrum (Signal Analytics, Vienna, VA).

Biological activity of HTSTS1-TM and HT84-TM in vitro

The in vitro biological activity of HTSTS1-TM- or HT84-TM-coated tumor cells was measured using a lymphocyte proliferation assay. PBL were isolated from the blood of healthy donors over a Histopaque 1077 gradient (Sigma) and aliquoted to 2 × 10{sup}6{sup} cells/well in 96-well U-bottom plates. The PBLs were incubated in 0.2 ml RPMI 1640 medium containing 5% FCS and irradiated (10,000 rad) coated or uncoated MA 148 cells at a 1:1 or 1:2 tumor cell to PBL ratio. MA 148 cells were coated with either 0.75 μM of HTSTS1-TM or HT84-TM, or serial 5-fold dilutions of HTSTS1-TM. After 72-h incubation (37°C, 5% CO2), cells were labeled with [3H]thymidine (1 μCi/well) for 18 h before harvesting. Incorporation of [3H]thymidine was determined using a liquid-scintillation counter.

Biological activity of HTSTS1-TM and HT84-TM in vivo

The in vivo biological activity of HTSTS1-TM- or HT84-TM-coated tumor cells was determined using an established immunization protocol (25–27). Five mice were in each treatment group. P815 cells (1 × 10{sup}6{sup} cells/ml) were incubated in serum-free medium alone or with HTSTS1-TM (0.17 or 1.67 μM) and injected into the flanks of B6D2F1 mice. Survival was determined daily for 15 days postimmunization.
mM) or HT84-TM (0.03, 0.17, or 0.85 mM) as described above. Tumor cells were then rinsed, resuspended at 1 × 10^7 cells/ml, and treated with 50 µg/ml mitomycin C (Mit C) for 1 h at 37°C. Mice were immunized s.c. in the foot with superantigen-coated or control tumor cells (1 × 10^7 cells in 50 µL/mouse). At 21 days postimmunization, antitumor immunity was determined by challenging mice intradermally in the axillary region with 5 × 10^3 or 1 × 10^4 viable P815 tumor cells. Previous studies using this tumor immunization model have shown that the primary P815 tumor-specific CTL response disappears by about 14–17 days, so that by 21 days a state of long-term memory immunity is present. Also, by waiting 21 days we reduce the possible contribution of nonspecific effects due to the superantigen. Biecting tumor diameters were measured with calipers every 2-3 days. Mice were sacrificed when tumor diameters exceeded 15 mm. All control mice developed progressively growing tumors. Some mice in the treatment groups did not, and of the tumors that did develop, some progressed and others regressed. Therefore, significant differences between control and test groups were determined using the nonparametric Wilcoxon Rank Sum test.

Results
Cloning and expression of HTSST1-TM and HT84-TM
The chimeric TSST1 or T84/c-erb-B-2 TM region DNA sequences were created by splice overlap extension (28) and subcloned into pET 17bH as described in Materials and Methods and depicted in Fig. 1A. Control of the chromosomally located T7 polymerase gene in E. coli host strain BL21(DE3) is not completely stringent, resulting in basal expression of proteins directed by the T7 promoter (33). Considerable levels of HTSST1 were expressed in BL21(DE3) without induction of T7 polymerase by IPTG (32). HTSST1-TM and HT84-TM are toxic to bacteria which express them, but can be successfully over-expressed if protein production is stringently repressed during growth. Expression of HTSST1-TM and HT84-TM was achieved when transforming the TSST1-TM/pET 17bH plasmids into E. coli host strain BL21(DE3) pLysS and growing cultures in 2%, rather than 0.4%, glucose (31). Successful expression of TSST1-TM and T84-TM under these more stringent growth conditions is shown on a Coomassie brilliant blue-stained 12% SDS-polyacrylamide gel of whole bacterial lysates from induced and uninduced cultures (Fig. 1B). Only IPTG-induced cultures over-expressed recombinant protein.

Purification of HTSST1-TM and HT84-TM
HTSST1-TM and HT84-TM were extracted from bacterial cell membranes using the nonionic detergent Triton X-100 (1%) because refolded preparations extracted using denaturing agents such as urea or guanidine-HCl did not induce proliferation. Protein in the clarified bacterial lysate loaded more efficiently onto the nikel-agarose resin when incubated batchwise. The resin was then thoroughly rinsed while on an immobilized support with a wash buffer containing glycerol. A rinse containing a comparatively high concentration of imidazole (100 mM) was necessary to elute host proteins nonspecifically associating with either the TM fusion proteins or the resin. Upon addition of 300 mM imidazole, HTSST1-TM or HT84-TM eluted between 4 and 20 ml. Positive fractions were pooled and dialysed against PBS. Fig. 1C depicts a representative Coomassie brilliant blue-stained 12% SDS-polyacrylamide gel showing purified HTSST1-TM and HT84-TM. This one-step purification strategy yielded per liter of bacterial culture 2 mg of HTSST-TM or 0.2 mg of HT84-TM with an average relative purity of 88%.

HTSST1-TM associates with MHC II-negative tumor cells
Superantigens normally bind MHC class II molecules on cells (6). The ability of HTSST1-TM to associate with cells through the TM region but not by MHC II binding was evaluated on four different MHC II-negative tumor cell lines: MA 148, RJ2.2.5, LLC, and EL4. RJ2.2.5 is a MHC II-negative mutant of the human B cell lymphoma, Raji (36). Murine cell lines EL4, LLC, P815 and the human ovarian carcinoma MA 148 are all MHC class II-negative, even after exposure to IFN-γ (data not shown; Ref. 37).

Spontaneous anchoring of HTSST1-TM onto the different tumor cell lines was determined by flow cytometry. The cells were incubated with HTSST1-TM or HTSST1 at 37°C, rinsed of unbound protein, and immediately prepared for flow cytometry. As shown in Fig. 2, HTSST1-TM spontaneously anchored onto all tumor cell lines tested. This association requires the TM region, because recombinant native HTSST1 did not bind to RJ2.2.5, MA 148, or LLC cells (Fig. 2). The cell lines tolerated serum deprivation variably, regardless of exposure to HTSST1-TM. Addition of serum
cells were coated with 1.67 μM HTSS1-TM or HT84-TM (10,000 events), LLC (7500 events), and EL4 (2000 events) were analyzed by flow cytometry using purified αT84 Ig, and FITC goat anti-rabbit Ig. Fluorescence was measured on cells exposed to serum-free medium (-), 0.75 μM HTSS1(-), or HTSS1-TM (——). HTSS1 binding to EL4 cells was not tested.

concentrations up to 2.5% did not interfere with HTSS1-TM association and improved viability. MA 148 cells prepared for flow cytometry 22 h after incubation with HTSS1-TM retained a fluorescence intensity at 25% the level of treated cells that were analyzed directly (data not shown). This is most likely due to internalization or shedding of HTSS1 into the medium, because preventing cell division with Mit C did not change this result. HTSS1-TM did not significantly associate with cells when incubated at 4°C (data not shown). To determine the distribution of HTSS1-TM association among coated cells and retention of cell integrity after incubation, anchored HTSS1-TM on MA 148 was also visualized by indirect immunofluorescence (Fig. 3).

MA 148 cells coated with HTSS1-TM or HT84-TM stimulate lymphocyte proliferation

The in vitro biological activity of HTSS1-TM- or HT84-TM-coated MA 148 cells was determined by lymphocyte proliferation assays. Fig. 4A shows the results of an experiment where MA 148 cells were coated with 1.67 μM of HTSS1-TM or HT84-TM before being cultured with PBLs. MA 148 cells coated with either HTSS1-TM- or HT84-TM-stimulated lymphocyte proliferation (Fig. 4A). To determine whether cells coated with smaller concentrations of protein were biologically active, MA 148 cells were coated with 5-fold serial dilutions of HTSS1-TM before being cultured with PBLs (Fig. 4B). Preincubating MA 148 cells with as little as 1 nM HTSS1-TM induced significant PBL proliferation vs uncoated MA 148 cells (p < 0.004, Fig. 4B). These results show that membrane bound HTSS1-TM and HT84-TM can induce lymphocyte proliferation in vitro.

Immunization with HTSS1-TM- or HT84-TM-coated P815 cells induces antitumor immunity

The in vivo biological activity of HTSS1-TM- or HT84-TM-coated murine P815 cells was determined using an established immunization protocol that measures a secondary response against parental tumor challenge (25–27). Mice (five per group) were immunized in the footpad with Mit C-treated P815 cells or P815 cells precoated with varying concentrations of HTSS1-TM or HT84-TM. We used P815 tumor cells for in vivo experiments because it is a well-characterized immunogenic tumor (38). Twenty-one days postimmunization, growth of parental tumor challenge was measured at a distant site. Mice preimmunized with either HTSS1-TM- or HT84-TM-coated P815 tumor cells exhibited significant antitumor immunity. Mice preimmunized with HTSS1-TM elicited a significant antitumor response in comparison to mice preimmunized with uncoated P815 tumor cells (p ≤ 0.03). The average diameters for this group reflect a reduced tumor incidence (three of five mice), delayed appearance of tumors that did develop, and partial regression of one tumor that eventually completely regressed (Fig. 5B). Mice preimmunized with P815 cells incubated with 1.67 μM of HTSS1-TM all initially developed tumor; however, the average value presented for this group reflects the complete regression.
of one tumor and the partial regression of two others (Fig. 5A). Because the higher dose of HTSST1-TM was less effective and because we challenged at a site distant from immunization after 21 days, the antitumor immunity observed was very likely tumor specific. The mice with tumors that completely regressed (one from each test group) were rechallenged with 1 x 10^6 parental P815 cells 87 days postimmunization and neither developed tumor (data not shown).

We show in Fig. 5C that HT84-TM also increased the immunogenicity of P815 tumor cells. Mice preimmunized with P815 cells incubated with 0.85 μM HT84-TM exhibited significant antitumor immunity in comparison to mice preimmunized with uncoated P815 tumor cells (p ≤ 0.02). Of the five mice in this group, one did not develop tumor and two developed tumors that completely regressed (Fig. 5D). All mice preimmunized with P815 cells incubated with 0.17 μM HT84-TM initially developed tumor, but two tumors completely regressed and one partially regressed (Fig. 5C). When comparing the antitumor responses elicited by equimolar concentrations of HT84-TM and HTSST1-TM (0.17 μM of HTSST1-TM or HT84-TM), HTSST1-TM-coated cells were more potent immunogens, correlating with the in vitro proliferation experiments (Fig. 4A). Together, these results suggest that HTSST1-TM- or HT84-TM-coated P815 tumor cells can stimulate systemic antitumor immunity. Optimizing the immunization protocol should further improve the antitumor immunity induced by HTSST1-TM- or HT84-TM-anchored tumor cells.

**Discussion**

In this study, we describe a novel and effective method for incorporating the superantigen TSST1, and perhaps other proteins, onto the surface of tumor cells. Fusing a TM sequence onto TSST1 resulted in its spontaneous association with four different tumor cell lines. Because the hydrophobic TM sequence mediates this passive anchoring, HTSST1-TM and HT84-TM should associate with membranes of most cells or tumors. Anchored HTSST1-TM and HT84-TM were biologically active in vitro, as shown by the ability of coated MA 148 cells to elicit proliferation of PBLs.

TSST1 is a powerful immunostimulant, and tumor cells coated with concentrations below the levels of detection still induced a biological response. Incubating concentrations of at least 25 nM HTSST1-TM could be detected on MA 148 cells by flow cytometry (data not shown). Yet coated MA 148 cells still stimulated significant PBL proliferation when incubated with HTSST1-TM concentrations 25-fold less than this. Incubating MA 148 cells with as little as 1 nM HTSST1-TM elicited a significant proliferative response in vitro (Fig. 4B). This result is not surprising when considering that soluble TSST1 concentrations as low as 2 pM will induce significant proliferation of human peripheral blood mononuclear cells in vitro (39).

Coating tumor cells with smaller concentrations of HTSST1-TM is not only more desirable for minimizing toxicity with the potential therapeutic application of this strategy but also resulted in better systemic antitumor immunity. Immunization with P815 tumor cells coated with 0.17 μM HTSST1-TM significantly inhibited subsequent onset and growth of a parental tumor challenge, whereas P815 cells incubated with 1.67 μM was less effective. It is not totally unexpected that P815 cells coated with higher concentrations of HTSST1-TM were less effective immunogens. Miethke et al. (40) have demonstrated a dose-response relationship between superantigen-induced T cell stimulation or inhibition in vivo, where increasing superantigen doses resulted in anergy and deletion. The ability of high doses of superantigen to cause T cell anergy or deletion in vivo has also been documented by others (41–44). Thus, P815 cells coated with the highest concentration of HTSST1-TM (1.67 μM) may have induced a diminished antitumor response because of T cell anergy or deletion. A possible mechanism for this effect might be the ability of TSST1 to directly stimulate macrophages through MHC II engagement (45–47). Excessive stimulation of macrophages has been shown to have immunosuppressive consequences (48), in part mediated by nitric oxide and TNF-α, that can interfere with a productive antitumor response (26, 49).
From a therapeutic perspective, we hypothesized that coating tumor cells with HTSST1-TM would focus a powerful T cell response to the context of the altered (and unaltered) cell while minimizing potentially toxic systemic exposure. However, immunologic processing and the intrinsic dynamic fluidity of cell membranes will make these imposed constraints inherently imperfect. Therefore, it is probable that in vivo both released and cell-bound HTSST1-TM contribute to the antitumor response. By coating tumor cells with HT84-TM, we sought to minimize cytotoxicity to neighboring MHC II-positive cells that could potentially be caused by dissociation of HTSST1-TM from the intact cell. That membrane-bound HTSST1-TM or HT84-TM contribute significantly to the in vivo antitumor response observed is indirectly demonstrated by the ability of HT84-TM-coated tumor cells to protect against subsequent tumor challenge. Unable to bind MHC II, free TSST188–194 should not encourage formation of a ternary primary signaling complex or the T cell costimulatory signals provided by APC contact and necessary for long-term immunity (23). Whereas 100-fold greater molar concentrations of free HT84 were required to elicit a response equivalent to HTSST1 in vitro (23), only 5-fold greater concentrations of membrane-associated HT84-TM were required to elicit a secondary response equivalent to HTSST1-TM in vivo.

An antitumor strategy that anchors a superantigen on MHC II-negative tumor cells assumes T cell stimulation, but which circumstances conventionally defined MHC “presentation.” The stimulatory mechanism likely involves a third party APC, which minimally would provide necessary costimulatory signals. Numerous studies have shown the ability of superantigens to elicit MHC II-independent T cell stimulation in vitro as long as costimulatory signals were provided (50–53). In addition, TSST1 promoted proliferation of isolated CD4 T cells in vitro when presented by immobilized mAbs against the MHC II binding region (54). Furthermore, it has been demonstrated in several reports that anchoring Staphylococcal enterotoxin A (SEA) onto MHC II-negative tumor cells through Abs directs T cell-mediated cytotoxicity against these tumors (55–58). These results suggest that superantigens can induce immunostimulation in the absence of MHC II molecules, and that artificially anchoring a superantigen onto a cell surface can substitute for MHC II presentation. That superantigens can induce MHC II-independent immunostimulation is therapeutically relevant. Because superantigens have been shown to generally direct T cell cytotoxicity against MHC II-positive cells on which they are bound (59), clinically feasible application of superantigen-based antitumor strategies will likely require compromising MHC II binding capabilities. Indeed, SEA mutants with diminished MHC II binding affinities have been tested in vitro and in vivo. A SEA mutant unable to engage MHC II molecules promoted cell-mediated cytotoxicity in vitro indistinguishably from native SEA when anchored onto cells with Ab (60). Tumor-bearing mice treated with this Ab-directed SEA mutant mounted antitumor responses with reduced toxicity (61). HT84-TM contains the TSST1 C-terminal residues 88–194 with amino acids critical for superantigenicity while lacking the MHC II binding domain (19–22). In vitro experiments show that soluble TSST188–194 expressed as a recombinant protein does not bind MHC II molecules (23). Therefore, HT84-TM should induce an antitumor response without binding MHC II molecules and with reduced cytotoxicity against normal MHC II-positive cells.

TSST1-TM fusion proteins represent a novel method for passively introducing immunostimulatory proteins onto cells.

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References


