Glycolipid-anchored IL-12 Expressed on Tumor Cell Surface Induces Antitumor Immune Response

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ABSTRACT

Systemic or local administration of cytokine has been used as a mode to enhance the antitumor immune response induced by many cancer vaccines. We have investigated whether the expression of cytokines on the tumor cell surface as a glycolipid (GPI)-anchored form will be effective in inducing antitumor immune response using a GPI-anchored interleukin (IL)-12 (GPI-IL-12) as a model. GPI-IL-12-induced proliferation of concanavalin A-activated T cells and induced IFN-γ secretion by activated and allogeneic T cells, indicating that the membrane-expressed IL-12 can stimulate T cells. GPI-IL-12 expressed on the tumor cell surface prevented tumor growth in mice in a highly tumorigenic murine mastocytoma model. These results suggest that the cell surface-expressed GPI-IL-12 can be effective in inducing antitumor immune response, and GPI-anchored cytokines expressed on the tumor cell surface may be a novel approach to deliver cytokines at the immunization site during vaccination against cancer. Furthermore, purified GPI-anchored cytokines can be used to quickly modify tumor membranes by the protein transfer method to express the desired cytokines for vaccine development.

INTRODUCTION

Cytokines play a crucial role in the induction of antitumor immune response (1–3). Preclinical studies have demonstrated that administration of cytokines such as IL3, IL4, IL-6, and IL-12 induce stimulation of antitumor immune responses in murine tumor models. However, systemic administration of cytokines such as IL-12 in human patients resulted in unwanted side effects (4). This was circumvented by transfer of cytokine genes into tumor cells for localized secretion of cytokines at the tumor microenvironment (1, 9). Tumors transfected with genes specific for cytokines such as IL-12 and GM-CSF have been shown to induce tumor-specific immunity (3, 9, 10). However, expression of cytokines by gene transfer procedure requires viral vectors and establishment of cell lines that are not desirable and are time consuming under clinical settings. Moreover, primary tumor cells are not very receptive for gene transfer. To circumvent these problems, many investigators have used different strategies, such as co-injecting tumors with fibroblasts secreting cytokines (11) or biodegradable gelatin polymers encapsulated with cytokines with tumor cell preparations (12). We have investigated whether the expression of cytokines, such as IL-12, on a tumor cell surface in GPI-anchored form will be effective in inducing antitumor immune response. This form of cytokine could be used in a protein transfer technique to modify tumor membranes for vaccine preparation (13). Moreover, membrane-associated cytokines may serve as a slow release depot at the immunization site.

In this study, we have attached a GPI anchor to both subunits of mouse IL-12 and expressed them on the tumor cell surface. Results show that the GPI-anchored IL-12 can stimulate T cells and can induce antitumor immune response in mice. These findings suggest that the expression of cytokines on tumor cell membranes may be an alternative approach to deliver cytokines for tumor vaccination.

MATERIALS AND METHODS

Cell Lines, mAbs, and Cytokines. Murine mastocytoma (P815) and rat hybridomas against murine MHC class I (M1/42), CD54 (YN1.1), CD80 (1G10), and CD24 (M1/69) were purchased from American Type Culture Collection (Manassas, VA). Rat antimurine IL-12 hybridomas (C15.6 and C17.8) were kind gifts from Dr. Trinchieri (Wistar Institute, Philadelphia, PA). P815 cells were cultured in DMEM (DMEM supplemented with 5% fetal bovine serum, 2 mM Glutamax I (Invitrogen, Carlsbad, CA), 1 mM sodium pyruvate, 100 units/ml penicillin, 100 µg/ml streptomycin, 55 µM β-mercaptoethanol, and 50 µg/ml gentamicin). The hybridomas were maintained in RPMI 1640 supplemented with 10% calf serum (HyClone, Logan, UT), 2 mM glutamine, and other additives at concentrations mentioned above (complete RPMI 1640). All cell culture reagents were purchased from Mediatech, Inc. (Herndon, VA), unless indicated. Unconjugated and horseradish peroxidase- or FITC-conjugated-F(ab′)2 goat antirat IgG and F(ab′)2 goat antirat IgG were purchased from Jackson Immunochemicals (West Grove, PA). Mouse antihuman IFN-γ mAbs (clones 2G1 and B133.5) were purchased from Pierce Endogen (Rockford, IL). Rat antiamurine IFN-γ mAbs (clones R4-6A2 and XMG1.2) were kind gifts from Dr. K. Ziegler (Emory University, Atlanta, GA).

Construction of GPI-IL-12 and secIL-12 cDNAs. A mammalian expression vector cassette with GPI-anchor signal sequence of CD59 (containing AflII linker at the 5′ end of CD59 cDNA) was constructed by cloning a truncated human CD80-CD59 cDNA in pcDNA3neo (Invitrogen) at EcoRV/ApaI sites (Fig. 1A). This expression vector cassette was used to make cDNAs encoding the GPI-anchored form of mouse IL-12 (GPI-IL-12). IL-12 is a disulfide-linked heterodimer consisting of p40 subunit (M35,000) and p35,000 (p40) polypeptides (9). The coding regions (excluding the stop codon) of both subunits of IL-12 were PCR amplified using pNGVL3-IL-12 cDNA as the template using Pfu DNA polymerase (Strategene, La Jolla, CA). The primers to amplify p35 cDNA were forward (catcaccagctctctca) and reverse (cattgcttaaggatcggaccctgcagggaa) primers were used to amplify cDNA encoding the GPI-anchored form of IL-12 (GPI-IL-12). IL-12 is a disulfide-linked heterodimer consisting of M₃₅₅₀₀₀ and M₃₄₄₀₀₀ polypeptides (₉). The coding regions (excluding the stop codon) of both subunits of IL-12 were PCR amplified using pNGVL3-IL-12 cDNA as the template using Pfu DNA polymerase (Strategene, La Jolla, CA). The primers to amplify p35 cDNA were forward (catcaccagctctctca) and reverse (cattgcttaaggatcggaccctgcagggaa) primers were used to amplify cDNA encoding the GPI-anchored form of IL-12. The reverse primers were designed to have an AflII linker (underlined). The truncated CD80 cDNA was excised from the pcDNA3-CD59-pcDNA3neo mammalian expression vector with EcoRV/AflII, which leaves the GPI-anchor addition signal sequence of CD59 with the vector cassette (Fig. 1A). The AflII-digested PCR products of p35 and p40 cDNAs were then cloned into the cassette containing a GPI-anchor addition signal sequence of CD59 at the EcoRV/AflII sites. Using this strategy, both p35-CD59 and p40-CD59 cDNAs were cloned into pcDNA3neo mammalian expression vector (Fig. 1A), and the p35-CD59 cDNA was further subcloned into the pUB66 vector (Invitrogen). cDNA encoding secIL-12 was mobilized from pNVL3-IL-12 and cloned into pUB66neo (pUB66neo/secIL-12) at KpnI and Apal sites.

Establishing Transfectants Expressing GPI-anchored or secIL-12. P815 transfectants expressing GPI-anchored IL-12 were established by transfecting murine p35-CD59-pUB66neo (10 µg) and p40-CD59-pcDNA3neo (10 µg) cDNAs by electroporation using a Bio-Rad gene pulser II (Hercules, CA). The electroporation was performed using the cells in serum-free RPMI 1640 pulsed at 960 µF and 0.25 kV/cm. After 48 h of transfection, the GPI-IL-12 cells were enriched by biomagnetic selection using anti-IL-12 mAb (C17.8) and...
sheep antirat IgG magnetic beads (10 beads/cell) and two cycles of a panning method as described earlier (14). The enriched GPI-IL-12 cells were cultured in cDMEM containing 10 g/ml blasticidin and 1 mg/ml G418. P815 cells secreting IL-12 (P815-secIL-12) were established by transfecting pUB6bla-secIL-12 cDNA. Cells secreting IL-12 were selected in cDMEM containing 10 g/ml blasticidin. Single-cell clones of P815-GPI-IL-12 and secIL-12 were established by limited dilution cloning. The uncloned and cloned P815-GPI-IL-12 transfectants were used in this study. To determine the cell surface expression of IL-12, MHC class I, CD54, CD80, and CD24 on uncloned and cloned populations, the cells were stained with the appropriate mAbs and analyzed using a FACScan flow cytometer (Becton-Dickinson, San Jose, CA). To confirm the GPI linkage of cell surface-expressed IL-12, cells were treated with PIPLC (14), followed by flow cytometric analysis. To determine the growth characteristics of GPI-IL-12 tumor cells in vitro, P815 or P815-GPI-IL-12 cells (1 x 10⁶) were cultured in cDMEM for 24 h at 37°C. The cells were then pulsed with [³H]thymidine (1 µCi/well) and incubated for another 18 h. [³H]Thymidine uptake was determined in a Packard top count scintillation counter.

Preparation of Isolated Membrane Vesicles from P815-GPI-IL-12 Transfectants. Isolated membranes were prepared from P815 and P815-GPI-IL-12 cells by sucrose gradient ultracentrifugation, as described earlier (13, 15). Membranes were resuspended in protein-free RPMI 1640 with antibiotics and frozen in aliquot at −80°C. Protein concentrations of membranes were determined by the Bio-Rad dye-binding method using BSA as a standard. The expression of GPI-IL-12 and other surface markers on the isolated membranes were determined by ELISA using appropriate mAbs, as described earlier (15).

To quantitate IL-12 expressed on isolated membranes, GPI-IL-12 isolated membranes (150 µg) were lysed in 20 mM Tris-HCl (pH 8.0) containing 1% octyl β-glucoside for 1 h and centrifuged at 20,000 x g for 1 h to collect clear lysate. IL-12 in the lysate was determined by sandwich ELISA using anti-IL-12 mAbs (C17.8 and biotinylated-C15.6) and horseradish peroxidase-conjugated avidin. Color was developed using 3,3',5,5'-tetramethylbenzidine as substrate, and the reaction was stopped with 2N H₂SO₄. The color that developed was read at 415 nm in an ELISA microplate reader (Molecular Devices, Sunnyvale, CA). Isolated membranes prepared from P815 cells were treated identically and used as a negative control.
Proliferation of PHA-activated Human T Cells and ConA-activated Murine Splenocytes. T cells were enriched from peripheral blood mononuclear cells isolated from healthy donors as described (15). PHA-activated human T cells were prepared using 1% PHA (Invitrogen) by standard procedure (16). P815 and P815-GPI-IL-12 cells (stimulators) were treated with 50 μg/ml mitomycin C for 30 min at 37°C, washed extensively with complete RPMI 1640, and used in the proliferation assays. PHA-activated T cells (responders) were cocultured with mitomycin C-treated stimulator cells for 72 h. Cells were pulsed with 1 μCi/well [3H]thymidine (Amersham, Arlington Heights, IL) for the final 18 h and harvested using a Packard filtermate cell harvester (Meriden, CT). [3H]Thymidine uptake was counted in a Packard top count microplate scintillation and luminescence counter (Downers Grove, IL). Similarly, proliferation of ConA-activated splenocytes (responder) was done by coculturing responders with mitomycin C-treated stimulator cells for 72 h. The uptake of [3H]thymidine after an 18-h pulse with [3H]thymidine (1 μCi/well) was determined as described above.

MLTR and IFN-γ Release Assay. An allogeneic MLTR assay was carried out to determine the efficacy of GPI-IL-12 to induce alloantigen-specific T-cell stimulation. Mitomycin C-treated P815 (H-2d) or P815-GPI-IL-12 cells were cocultured for 72 h with unactivated splenocytes of C57BL/6 (H-2b) mice. Murine rsIL-12 was included as a positive control. The MLTR cultures were centrifuged, and the supernatant was analyzed for the release of IFN-γ to determine the IL-12-dependent T-cell stimulation. IFN-γ release was determined by sandwich ELISA using corresponding mAb pairs. Similarly, to determine the IL-12-dependent stimulation of activated T cells, P815-GPI-IL-12 cells or membranes isolated from P815-GPI-IL-12 cells were cocultured with ConA-activated mouse splenocytes or PHA-activated human T cells as responders. The release of IFN-γ by activated T cells was used as a measure to determine the IL-12-dependent T-cell stimulation. Supernatants were collected after 48 h, and the release of human or murine IFN-γ was determined by sandwich ELISA using paired mAbs.

Tumor Challenge Studies. Female DBA/2 mice (6–8 weeks) were purchased from the Jackson Laboratory (Bar Harbor, ME) and maintained in the Emory University animal facility according to the regulations of the Institutional Animal Care and Use Committee. Mice (5–10 mice/group) were challenged (s.c.) with P815 or P815-GPI-IL-12 or P815-seclIL-12 cells (5 × 10^5 cells/mice) and were monitored twice a week for tumor growth. Two measurements of tumors that are perpendicular to each other were measured using vernier calipers. Tumor size (mm^3) was quantitated by multiplying the two diameters for each mouse in control and experimental groups. Mice were euthanized when tumor size reached >2 cm. To determine the presence of IL-12 in the systemic circulation, 3 mice/group received injections of serum-free RPMI 1640 or live P815, P815-GPI-IL-12, or P815-seclIL-12 cells (5 × 10^5 cells in 200 μl of serum-free RPMI). Serum samples were collected and pooled (3 mice/group), and IL-12 and IFN-γ in serum samples were quantitated by sandwich ELISA using appropriate mAbs.

RESULTS

Chimeric IL-12-CD59 Is Expressed on the Cell Surface as a GPI-anchored Protein. The cDNAs encoding the entire coding region of the p35 and p40 subunits of mouse IL-12 were ligated in-frame to a GPI-anchor addition signal sequence of CD59 in a mammalian expression vector cassette (Fig. 1A). Stable transfectants of a murine mastocytoma, P815, expressing mouse GPI-IL-12 were established by cotransfecting chimeric cDNAs of p35 and p40 subunits, as described in “Materials and Methods.” Flow cytometric analysis of the P815-GPI-IL-12 transfectants showed cell surface expression of IL-12 (Fig. 1B). In addition, the expression of other cells surface markers, such as MHC class I, was not altered in these transfectants, as compared with the P815 cells (Fig. 1B). More than 90% of the GPI-IL-12 protein expressed on transfected cells was released by PIPLC treatment (Fig. 1B), indicating that the IL-12 is anchored to the cell surface via a GPI moiety.

Cell Surface-expressed GPI-IL-12 Induces T-Cell Proliferation. Murine rsIL-12 has been shown to stimulate activated human and murine T cells (16). Therefore, the functional integrity of GPI-IL-12 was determined for its ability to induce the proliferation of activated T cells. PHA-activated human T cells were cocultured with mitomycin C-treated P815-GPI-IL-12 cells. GPI-IL-12+ cells induced T-cell proliferation, and levels of proliferation were similar to those obtained using 0.5 ng/ml of rsIL-12 (Fig. 2A). Similarly, the ability of GPI-IL-12+ cells to induce the proliferation of ConA-activated murine splenocytes was also determined. P815-GPI-IL-12 cells were able to induce proliferation of ConA-activated splenocytes over P815 cells (Fig. 2B). It has been shown that some GPI-anchored proteins, such as CD16B, are released from the cell surface (17). Therefore, to determine whether the induction of T-cell proliferation was mediated by the cell surface-expressed GPI-IL-12 and not because of shedding or secretion of IL-12, P815 and P815-GPI-IL-12 cells were cultured in cDMEM, and supernatants were collected after 48 h. Supernatants were centrifuged at 100,000 × g to remove any membrane fragments.
or particulate materials and tested for the presence of IL-12 in a T-cell proliferation assay using PHA-activated human T cells. As shown in Fig. 2C, mitomycin C-treated P815-GPI-IL-12 induced proliferation of PHA-activated human T cells, whereas P815 or P815-CD86 cells did not. However, under similar assay conditions, the supernatant obtained from P815-GPI-IL-12 cells did not induce proliferation, suggesting that there is no detectable level of IL-12 released into the supernatant from P815-GPI-IL-12 cells. These findings indicate that GPI-IL-12 is expressed as a functionally active heterodimer on the cell surface.

GPI-IL-12 Induces the Release of IFN-γ by Activated Splenocytes. It has been well established that IL-12 can stimulate T and natural killer cells and induce the release of Th1-type cytokines such as IFN-γ (9). Therefore, the ability of the cell surface-expressed GPI-IL-12 in inducing the release of IFN-γ was tested. P815 cells did not induce IFN-γ release from the activated cells. However, coculturing P815-GPI-IL-12 cells induced IFN-γ release by ConA-activated splenocytes (Fig. 3A).

Augmentation of Allogeneic T-Cell Stimulation by GPI-IL-12. The induction of allogeneic T-cell stimulation by GPI-IL-12 was determined in a MLTR assay. Unactivated splenocytes from C57BL/6 mice (H-2b/) were cocultured with mitomycin C-treated P815 (H-2b/) or P815-GPI-IL-12 cells. IFN-γ released by the stimulated allogeneic splenocytes was measured to determine the IL-12-dependent T-cell stimulation. The addition of P815-GPI-IL-12 cells induced the release of IFN-γ as compared with P815 control (Fig. 3B). Similar levels of IFN-γ were observed when allogeneic splenocytes were cocultured with P815 cells mixed with rsIL-12. Under similar conditions, very low levels of IFN-γ release by allogeneic T cells were seen in the presence of rsIL-12 alone, and P815 cells did not induce the release of IFN-γ. These findings indicate that the increased release of IFN-γ seen with P815-GPI-IL-12 is attributable to augmentation of alloantigen-mediated T-cell stimulation.

Membrane Vesicles Isolated from P815-GPI-IL-12 Cells Induce Release of IFN-γ by Activated T Cells. A potential application of making GPI-anchored cytokines such as GPI-IL-12 is that the purified GPI-IL-12 can be used to modify isolated tumor membranes for vaccine preparation by a protein transfer approach (13, 15). Moreover, the isolated tumor cell membranes expressing GPI-IL-12 can also be used as a vaccine for intratumoral administration. Therefore, the isolated cell membranes were prepared from P815-GPI-IL-12 cells and determined whether it can induce stimulation of activated T cells. The isolated membranes showed the expression of GPI-IL-12 and other surface markers such as MHC class I and CD54 (data not shown). The release of IFN-γ by ConA-activated murine splenocytes and PHA-activated human T cells was used as a measure to determine the IL-12-dependent T-cell stimulation. The addition of GPI-IL-12−/− isolated cell membranes in the proliferation assay resulted in the release of IFN-γ by ConA-activated splenocytes. The level of IFN-γ release induced by the GPI-IL-12−/− isolated cell membranes is comparable with that seen with rsIL-12. Membranes prepared from P815 cells did not induce the release of IFN-γ from the activated cells. Similarly, membranes prepared from P815-GPI-IL-12 cells also showed an increase in IFN-γ release by PHA-activated human T cells (data not shown). These findings indicate that the isolated membranes expressing GPI-IL-12 retained its functional activity to stimulate activated T cells.

Antitumor Immune Response Induced by GPI-IL-12 Expressed on Tumor Cells. Before using the P815-GPI-IL-12 transfectants in animal studies, the growth characteristics of P815-GPI-IL-12 cells in vitro were determined in a proliferation assay as described in “Materials and Methods.” The basal proliferation of P815 and P815-GPI-IL-12 cells were similar (data not shown), indicating that transfecting GPI-IL-12 into mastocytoma cells did not change the growth characteristics of the cells in vitro. The ability of cell surface-expressed GPI-IL-12 to induce antitumor immune response in vivo was determined using a highly tumorigenic and moderately immunogenic mastocytoma tumor model. Mice were inoculated with live P815 or P815-GPI-IL-12 cells and monitored for tumor development and survival. To compare the efficiency of secretory versus GPI-anchored IL-12 in inducing an antitumor response tumor, studies were done using P815 cells expressing GPI-IL-12 (uncloned cells established by panning) or cloned P815-GPI-IL-12 or P815-secIL-12 cells. The mice inoculated with control P815 cells developed tumors by day 10, and
The tumors grew progressively (Fig. 4A). All of the mice in this control group were either dead or euthanized (when the tumors reached the allowed limit), 44 days after inoculation of P815 cells (Fig. 4B). However, all of the mice inoculated with uncloned P815-GPI-IL-12 cells survived and were tumor free up to 55 days (Fig. 4). Tumors developed only after 55 days of tumor inoculation in 40% of mice, and all of the mice in this group developed tumors by day 80. Interestingly, all of the mice inoculated with cloned P815-GPI-IL-12 or P815-secIL-12 cells were tumor free even after 75 days (Fig. 4). To determine whether the tumors developed in mice that received injections of uncloned P815-GPI-IL-12 cells still express transfected-GPI-IL-12 in the absence of selection pressure, tumors were excised from one of the mice challenged with P815-GPI-IL-12 cells. Tumor cells were isolated by collagenase and dispase treatment, and the cell surface expression of IL-12 and other antigens were determined by flow cytometry. The expression levels of MHC class I and CD54 were not altered; however, the expression of GPI-IL-12 was completely lost in these tumor cells (data not shown).

Next, we determined whether the antitumor immune response induced by GPI-IL-12 is attributable to either systemic or local effect. Mice were inoculated with live P815-GPI-IL-12 or P815-secIL-12 or wild-type P815 cells or RPMI 1640 medium alone. Serum samples were collected for 3 days, and serum IL-12 and IFN-γ levels were estimated by sandwich ELISA. There was no difference in serum IL-12 levels between mice that received injections of P815-GPI-IL-12 or P815 cells or RPMI 1640 medium. However, under identical conditions, the serum IL-12 levels were increased ~2-fold after 3 days in mice that received injections of P815-secIL-12 cells (data not shown). We have also determined the serum IFN-γ as a measure of IL-12 in the systemic circulation of these mice. Serum IFN-γ levels were the same in mice that were given injections of P815-GPI-IL-12 or P815 cells or RPMI 1640 medium alone (data not shown). However, a time-dependent increase in IFN-γ (2- and 4-fold at days 2 and 3 after inoculation, respectively) was seen in mice that received injections of P815-secIL-12 cells. These findings suggest that the antitumor immune response induced by GPI-IL-12 may be mediated by local effect, whereas secIL-12 may act through entering the systemic circulation. However, more detailed studies are needed to address the exact mechanism(s) of antitumor immune response induced by GPI-IL-12.

**DISCUSSION**

Recently, we have used a novel protein transfer approach using GPI-anchored costimulatory cell adhesion molecules to develop cancer vaccines (13, 15). GPI-anchored proteins have a unique property to intercalate spontaneously into the outer leaflet of the lipid bilayer. With the advent of recombinant technique, we (14) and others (18) have converted transmembrane proteins to GPI-anchored cell surface proteins. Using this approach, EG7 tumor membranes modified with GPI-CD80 have been shown to induce a potent antitumor immune response in the murine thymoma model (13), suggesting that tumor membranes modified with GPI-anchored costimulatory molecules could be used as an effective cancer vaccine.

The present study investigated whether introducing a cytokine on tumor membranes as a GPI-anchored form can induce antitumor immune response. In this study, IL-12 was used as a model cytokine to determine whether the membrane-expressed GPI-anchored form of cytokine can induce an antitumor immune response. IL-12 is known to activate and enhance the development of antigen-specific CTLs (9) and can also attract inflammatory cells, natural killer cells, T cells, and other antigen-presenting cells to the vaccination site for a potent immune response (9). Moreover, expression of IL-12 has been shown to induce antitumor immunity in many tumor systems (11, 19, 20). The study presented here shows that GPI-anchored IL-12 is fully functional in inducing the proliferation of activated T cells and induction of IFN-γ production by T cells. Our results also show that cell surface expression of IL-12 as a GPI-anchored form on mastocytoma completely protected the mice up to 55 days when challenged with uncloned cells. However, tumors developed after 55 days. Interestingly, all of the mice inoculated with cloned P815-GPI-IL-12 or P815-secIL-12 cells were tumor free up to 95 days. This suggests that the tumor formation after 55 days in mice that received the injections of uncloned P815-GPI-IL-12 cells may be attributable to antigenic heterogeneity in cultured P815 cells. Using this tumor system, it has been shown that after nearly complete rejection, P815 tumors reappear after 55 days, and this is because of the emergence of escape variants with the loss of CTL epitopes (21). Interestingly, analysis of surface markers on tumor cells excised from the tumors that appeared after 55 days showed that these tumors lost the expression of surface IL-12 (data not shown). This could be attributable to the lack of a selection marker for the transfectants in vivo. At present it is not clear whether reappearance of the tumors seen in our studies after an initial protection in an uncloned population is attributable to one or a combination of the following mechanisms: (a) the loss of IL-12 expression on tumor cells; (b) the loss of antigenic epitopes; and/or (c) a short-lived memory response induced by IL-12-expressing tumors. Additional studies are necessary to address these possibilities.

Studies have shown that other cytokines such as IL-2, IL-3, IL-4,
and GM-CSF (22–24) are effective in stimulating immune cells when they are expressed on cell membranes. Tumors transfected with polypeptide membrane-anchored GM-CSF induced an antitumor immune response in mice (24). The advantage of GPI-anchored cytokines over polypeptide-anchored cytokines is that the isolated tumor cell membranes can be modified within 2–4 h to express the desired cytokines by protein transfer for administration in clinical settings (13, 15). This procedure does not involve any viral vectors or live cells. Recently, we have shown that membranes prepared from surgically removed human tumor tissue can be modified to express GPI-anchored costimulatory adhesion molecules (15). Furthermore, isolated membranes that were frozen for 2 years can also be modified to express the desired GPI-anchored costimulatory molecules by protein transfer (15). Such modified membranes efficiently provided costimulation for T-cell proliferation in vivo and in vitro (13, 15). Apart from the advantage of GPI-anchored cytokine use in protein transfer studies, isolated tumor membranes expressing GPI-anchored cytokines may also form a slow release depot at the immunization site. Furthermore, GPI-anchored cytokines incorporated onto cell membranes can form an insoluble depot at the immunization site and may last longer as compared with soluble cytokines. The availability of cytokines at the immunization site will bring immune cells to the vaccination site for better antigen uptake and stimulation, thus increasing the efficiency of cancer vaccines. The tumor membranes modified with GPI-anchored cytokines thus provide an alternative form of cytokine delivery to enhance antitumor immune response.

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