HLA Tetramer–Based Artificial Antigen-Presenting Cells Efficiently Stimulate CTLs Specific for Malignant Glioma

Xiaobing Jiang,1 Xiaoling Lu,2 Ruen Liu,3 Fangcheng Zhang,1 and Hongyang Zhao1

Abstract

Purpose: The interleukin-13 receptor α2 (IL-13Rα2) is a glioma-restricted cell-surface epitope not otherwise detected within the central nervous system. Here, we report a novel approach for targeting malignant glioma with IL-13Rα2–specific CTLs.

Experimental Design: Artificial antigen-presenting cells (aAPC) were made by coating human leukocyte antigen (HLA)-A2/pIL-13Rα22345-354 tetrameric complexes, anti-CD28 antibody, and CD83 molecules to cell-sized latex beads, and used to stimulate IL-13Rα2–specific CTLs from the peripheral blood mononuclear cells of HLA-A2+ healthy donors. After multiple stimulations, the induced CTLs were analyzed for tetramer staining, IFN-γ production, and CTL reactivity.

Results: Tetramer staining assay showed that the induced CTLs specifically bound HLA-A2/pIL-13Rα22345-354 tetramers. The CTLs specifically produced IFN-γ in response to the HLA-A2/pIL-13Rα22345-354–aAPCs and exhibited specific lysis against T2 cells pulsed with the peptide pIL-13Rα22345-354 and HLA-A2+ glioma cells expressing IL-13Rα22345-354, whereas HLA-A2+ glioma cell lines that express IL-13Rα22345-354 could not be recognized by the CTLs. The peptide-specific activity was inhibited by anti–HLA class I monoclonal antibody.

Conclusion: The induced CTLs specific for IL-13Rα22345-354 peptide could be a potential target of specific immunotherapy for HLA-A2+ patients with malignant glioma.

Despite significant advances in modern microsurgery, radiotherapy, and chemotherapy, the prognosis for patients with malignant glioma remains poor (1). Therefore, the development of a new treatment modality is needed. Although the central nervous system (CNS), and tumors that arise therein, reside in an "immunologically privileged" site (2), many data have successfully shown that effective anti-CNS tumor immune responses can be induced in preclinical models using syngeneic tumor- and dendritic cell–based vaccines (3–5). In addition, activated, antitumor immune cells have the potential to migrate into the CNS and to selectively destroy malignant cells that have infiltrated normal CNS tissues (6). Therefore, immunotherapy for glioma is an attractive alternative treatment option.

The induction of antigen-specific CTLs has been suggested to be highly efficacious in the prevention and treatment of various types of tumors (7). CTLs recognize "processed" peptides that are derived from endogenous proteins and presented on the cell surface in association with MHC class I molecules (7, 8). Peptides that bind to a given MHC class I molecule have been shown to share common amino acid motifs, which are called major anchor motifs (8). Hence, tumor-specific CTLs can recognize and select the antigenic peptides by scanning peptide sequences, and then kill tumor cells in an antigenic peptide-specific fashion. Interleukin-13 receptor α2 (IL-13Rα2) chain has been reported to be abundantly and specifically overexpressed in malignant glioma and not otherwise presented in the normal brain (9–11). IL-13Rα2–specific CTLs can be generated in vitro using peptide-pulsed autologous dendritic cells as antigen-presenting cells (APC; ref. 12). However, this method is time-consuming and expensive due to the limited availability of donor-derived dendritic cells. To date, several reports indicated effective stimulation of T cells by human leukocyte antigen (HLA)-peptide ligand coating on aAPCs, such as liposomes (13, 14) and microbeads (15–18). The aAPCs simulate the natural APCs (such as dendritic cells, macrophages, and B cells), which are able to provide the dual signals (the antigen-specific and costimulatory signals) for T-cell activation. These aAPCs can be prepared by coating the T-cell receptor ligand (peptide-MHC) and costimulatory molecules (such as B7) on a cell-sized bead, and it provides a practical and convenient approach to generating antigen-specific T cells for adoptive immunotherapy.

In this study, we explored a simple and efficient method to induce IL-13Rα22345-354–specific CTLs from the peripheral blood mononuclear cells (PBMC) of HLA-A2+ normal donors using aAPCs made by coating HLA-A2/pIL-13Rα22345-354...
complexes, anti-CD28 antibody, and CD83 to cell-sized latex microbeads in vitro, in which these CTLs kept their antigen-specific cytolytic properties.

**Materials and Methods**

**Cell line and cell culture.** T2 cells were kindly provided by Prof. Nicholas Zavazava (Kiel University, Kiel, Germany). This cell bears the HLA-A*0201 gene, but expresses a very low level of cell-surface HLA-A2.1 molecules and is unable to present endogenous antigens due to a deletion of most of the MHC class II region, including the transporter associated with antigen processing and genes encoding for immunoproteasomal subunits. This cell line was maintained in RPMI 1640 (Invitrogen) supplemented with 10% fetal bovine serum (HyClone) and antibiotics. Glioma cell lines U251 (HLA-A2*, IL-13Rα2*) and A172 (HLA-A2*, IL-13Rα2*) were purchased from the American Type Culture Collection. These glioma cell lines were cultured in DMEM supplemented with 10% fetal bovine serum and antibiotics.

**Synthetic peptides.** The following HLA-A2–restricted peptides were used in this study: the IL-13Rα2–derived peptide WLPFGFIH (referred to as pIL-13Rα2F354–12) and the control peptide HIV-Gag peptide SLYNTVATL (pHIV; ref. 19). Peptides were synthesized by standard solid-phase chemistry and characterized by mass spectrometry. The purity of the synthetic peptides was >95% as indicated by analytical HPLC. Lyophilized peptides were dissolved in DMSO and stored at -80°C after dilution in PBS.

**Detection of IL-13Rα2 gene expression in glioma cell lines by reverse transcription-PCR.** The expression of IL-13Rα2 mRNA in glioma cell lines U251 and A172 was detected with reverse transcription-PCR. The primers used for amplification of human IL-13Rα2 and GAPDH were as follows: IL-13Rα2, 5′-TCTGCCCAGGAACTTTGAAC-3′ (S) and 5′-TCTGCCCAGGAACTTTGAAC-3′ (AS); GAPDH, 5′-ACAGTCAGCCG-CATCCTTC-3′ (S) and 5′-ITATTTGAGCGGATCTCG-3′ (AS). The PCR reactions using Taq DNA polymerase (Life Technologies) were subjected to 30 amplification cycles; conditions consisted of 94°C denaturation for 30 s, 57°C primer annealing for 30 s, followed by primer extension at 72°C for 1 min.

**Preparation of HLA-A2/pIL-13Rα2F354 tetramers.** Synthesis of HLA-A2/pIL-13Rα2F354 tetramers was carried out according to the protocol of Altman et al. (20). Briefly, plasmids encoding HLA-A*0201 (heavy chain) molecules with a COOH-terminal biotinylation site and human β2m molecule were constructed by insertion of the target genes into pET28a. The heavy chain and β2m molecules were expressed in Escherchia coli, purified from inclusion bodies, and then refolded in the presence of excess pIL-13Rα2F354 to form the HLA-A2/pIL-13Rα2F354 monomeric product. The folded product was then subjected to enzymatic biotinylation by BirA enzyme (Avidity) at 25°C for 12 h. This biotinylated HLA-A2/pIL-13Rα2F354 monomer was used for the preparation of the HLA-A2/pIL-13Rα2F354 tetramer. The HLA-A2/pIL-13Rα2345-354 tetramer was produced by mixing the purified biotinylated monomer with FITC-labeled streptavidin (Sigma) at a molar ratio of 4:1. HLA-A2/pHIV tetramer was also prepared according to the manufacturer’s protocol.

**Generation of HLA-A2/pIL-13Rα2F354–aAPCs.** The 5-μm sulfamate polystyrene latex (interfacial Dynamics) was incubated sequentially with streptavidin (1 μg/106 beads; Sigma), CD28-specific antibody, and CD83 (1 μg and 1.5 μg/106 beads, respectively; BD PharMingen) for 30 min in 1-mL PBS per 106 beads at 4°C on a rotator. The beads were then incubated with biotinylated HLA-A2/pIL-13Rα2F354 complexes (2 μg/106 beads) for 1 h at 4°C on a rotator. After each incubation step, the beads were washed with PBS. The HLA-A2/pIL-13Rα2F354–aAPCs were stored in PBS at 4°C.

**Induction of CTL.** PBMCs from five healthy HLA-A2* donors were separated using standard Ficoll-Hypaque (Sigma) gradient density centrifugation. The PBMCs were stimulated with HLA-A2/pIL-13Rα2F354–aAPCs in vitro using the protocol adapted from previous studies (21). Briefly, The PBMCs were used as responder cells and stimulated with HLA-A2/pIL-13Rα2F354–aAPCs. Responder cells (3 × 106 per well) were cocultured with HLA-A2/pIL-13Rα2F354–aAPCs (3 × 106 per well) in 24-well plates in RPMI 1640 supplemented with 10% fetal bovine serum (1 mL/well). Interleukin-7 (IL-7, 10 ng/mL, PeproTech EC) was added on day 1. IL-2 (50 units/mL, R&D Systems) was added on day 4. Medium, IL-2, and IL-7 were replenished twice a week. On day 7 and weekly thereafter, responder cells were collected, counted, and replated at 3 × 106 per well, together with 3 × 105 HLA-A2/pIL-13Rα2F354–aAPCs per well in RPMI 1640 with 10% fetal bovine serum, 10 ng/mL IL-7, and 50 units/mL IL-2. After a total of four rounds of stimulations in vitro, responder cells were tested for tetramer staining and cytotoxicity assay.

**Tetramer staining.** Tetramer staining was done as previously described (22, 23). In brief, 1 × 106 cells were incubated in 100-μL fluorescence-activated cell sorting staining buffer (PBS supplemented with 1% bovine serum albumin and 0.05% NaN3) with 20 μg/mL HLA-A2/peptide tetramer at 37°C for 30 min. Cells were washed with PBS and subsequently incubated with phycoerythrin-Cy5–labeled anti-CD8 antibody (BD Pharmingen) at 4°C for 30 min. All cells were washed with PBS twice after being stained, and then fixed in 1% formaldehyde. Stained cells were analyzed with FACScanibur (Becton Dickinson).

**ELISA.** To determine the frequency of T cells capable of responding to a specific stimulus by secretion of IFN-γ, an enzyme-linked immunospot (ELISPOT) assay was done. The HLA-A2/pIL-13Rα2F354–aAPCs were used as stimulating target cells. Beads pulsed without HLA-A2/pIL-13Rα2F354 complexes were used as the negative control. Responder cells were cocultivated with the target cells at a stimulator cell-to-responder cell ratio of 1:10 at 37°C for 24 h in 96-well ELISPOT plates. The assay was done and developed according to the manufacturer’s instructions (BD Pharmingen).

**Cytotoxicity assay.** To analyze the cytotoxic activity of the HLA-A2/pIL-13Rα2F354–aAPCs–induced CTLs against various target cells, cytotoxic assays were done by incubating 113-Cr-labeled (500 μCi) target cells with effector cells at various effector/target ratios at 37°C for 4 h. Glioma cell lines U251 (HLA-A2*, IL-13Rα2*) and A172 (HLA-A2*, IL-13Rα2*), K562, and T2 cells pulsed without a peptide were selected as target cells. Other target cells, T2pIL-13Rα2F354 (pIL-13Rα2F354–pulsed T2 cells) and T2pHIV (pHIV-pulsed T2 cells), were prepared by incubating T2 cells with pHIV (50 μg/mL) and pHIV (50 μg/mL) for 3 h at 37°C. The percentage of 113-Cr release was calculated according to the following formula: 100 × (experimental release - spontaneous release) / (maximum release - spontaneous release).

**Inhibition of the cytotoxicity with HLA class I–specific monoclonal antibody.** T2pIL-13Rα2F354 and U251 target cells were incubated with anti–HLA class I monoclonal antibody (mAb) W6/32 (American Type Culture Collection); ref. 24) and a control isotypic mAb of irrelevant specificity IgG2 (BD Pharmingen) at a final concentration of 30 μg/mL for 40 min at 4°C before cytotoxicity assay. After incubation, the target cells were mixed with effector cells for the 113-Cr release assay.

**Statistical analysis.** All data in this study were analyzed using SPSS version 11.0 software (SPSS). P < 0.05 was considered as statistically significant.

**Results**

**IL-13Rα2 gene expression and HLA class I typing in glioma cell lines.** IL-13Rα2 mRNA expression was detected in U251 and A172 cell lines. The results of HLA class I typing detection

---

4 http://www.atcc.org/
showed that U251 was a HLA-A2+ cell line and A172 was a HLA-A2- cell line.

**Growth kinetics of coculture bulk and phenotype of induced T cells.** Following the first stimulation using HLA-A2/pIL-13Ro2345-354-aAPCs, PBMCs were expanded continuously. After 4 weeks of coculture with HLA-A2/pIL-13Ro2345-354-aAPCs, we could obtain as many as $1.5 \times 10^8$ to $1.8 \times 10^8$ T cells from an initial number of $3 \times 10^5$ cells, corresponding to a mean expansion of 50- to 60-fold. The phenotype of the expanded cells was measured by flow cytometry. The percentage of CD4+ cells gradually decreased whereas the CD8+ cells increased with the progression of the coculture with HLA-A2/pIL-13Ro2345-354-aAPCs. The phenotypes of the CTLs for pIL-13Ro2345-354 were CD8+, CD4+, CD3+, CD16+, and CD56+.

**Frequency of pIL-13Ro2345-354-specific CTLs increased by coculture with HLA-A2/pIL-13Ro2345-354-aAPCs as determined by tetramer staining.** A flow cytometric analysis of PBMCs was done before or after coculture with the HLA-A2/pIL-13Ro2345-354-aAPCs. Before the four rounds of stimulation using HLA-A2/pIL-13Ro2345-354-aAPCs, the frequency of CD8+ T cells stained with HLA-A2/pIL-13Ro2345-354 tetramers was 0.11%. However, after stimulation, fluorescence-activated cell sorting analysis revealed that 14.7% of CD8+ T cells were stained with HLA-A2/pIL-13Ro2345-354 tetramers, which was not observed when staining with the control tetramers (HLA-A2/pHIV tetramer; Fig. 1). The pIL-13Ro2345-354—specific CTLs expanded by HLA-A2/pIL-13Ro2345-354-aAPCs from the other four normal donors showed similar results (Table 1).

**ELISPOT assay.** The ELISPOT assay was used to determine the frequency of individual antigen-specific IFN-γ-producing T cells. As shown in Fig. 2, priming with HLA-A2/pIL-13Ro2345-354-aAPCs resulted in the generation of peptide-specific IFN-γ—producing CD8+ T cells after the stimulation. The average spot number of responding lymphocytes induced by HLA-A2/pIL-13Ro2345-354-aAPCs (164 ± 35 peptide-specific spots/10⁵ cells) was significant higher than that of responding lymphocytes induced by beads without HLA-A2/pIL-13Ro2345-354 (41 ± 8 peptide-specific spots/10⁵ cells; $P < 0.05$).

**Cytotoxicity of pIL-13Ro2345-354-specific CTLs.** The cytotoxic activity of the HLA-A2/pIL-13Ro2345-354-aAPCs—induced CTLs against various target cells was tested with the $^{51}$Cr-release assay. The CTLs exhibited ~70% specific lysis against T2pIL-13Ro2345-354 and U251 (HLA-A2+, IL-13Rα2+) at an effector/target ratio of 50:1. However, the CTLs showed an ~10% cytolysis against T2pHIV, A172 (HLA-A2+, IL-13Rα2), K562, and the T2 without a pulsed peptide at the same effector/target ratio (Fig. 3A). The specific killing activity of the CTLs induced by the HLA-A2/pIL-13Ro2345-354-aAPCs against specific target cells was much more obvious than in any other control group ($P < 0.05$). The specific CTLs for pIL-13Ro2345-354 induced by HLA-A2/pIL-13Ro2345-354-aAPCs from the other four normal donors showed similar specific lysis (Table 1). This result shows that the cytotoxicity of the HLA-A2/pIL-13Ro2345-354—aAPCs—induced CTL is pIL-13Ro2345-354 specific.

### Table 1. Induction of CTLs specific for pIL-13Ro2345-354 in four HLA-A2+ healthy donors

<table>
<thead>
<tr>
<th>Donor</th>
<th>Tetramer staining (%)</th>
<th>Cytotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HLA-A2/pHIV</td>
<td>HLA-A2/pIL-13Ro2345-354</td>
</tr>
<tr>
<td>1</td>
<td>0.14</td>
<td>15.9</td>
</tr>
<tr>
<td>2</td>
<td>0.17</td>
<td>16.4</td>
</tr>
<tr>
<td>3</td>
<td>0.09</td>
<td>14.6</td>
</tr>
<tr>
<td>4</td>
<td>0.08</td>
<td>13.8</td>
</tr>
</tbody>
</table>

Note: The CTLs induced by the HLA-A2/pIL-13Ro2345-354-aAPCs for 28 d were tested for tetramer staining and cytotoxicity activity. Tetramer-staining cells indicate the percentage of viable CD8+ HLA-A2/pIL-13Ro2345-354 tetramer—positive or CD8+ HLA-A2/pHIV tetramer—positive lymphocytes in the total population of viable lymphocytes; lysis indicates the percent lysis of pIL-13Ro2345-354—pulsed T2 cells (T2pIL-13Ro2345-354), U251 (HLA-A2+, IL-13Rα2+), or pHIV-pulsed T2 cells (T2pHIV). Results are shown for an effector/target ratio of 50:1.
Inhibition of cytotoxicity of HLA-A2/pIL-13Rα245-354-aAPCs–induced T cells by HLA class I–specific mAb (W6/32).

To determine whether the induced CTLs could recognize the specific target cells in a HLA class I–restricted manner, anti–HLA class I mAb W6/32 was used to block the cytotoxicity of the HLA-A2/pIL-13Rα245-354-aAPCs–induced CTLs. The cytotoxic activity against T2pIL-13Rα245-354 and U251 was significantly eliminated by W6/32. As shown in Fig. 3B and C, incubation of T2pIL-13Rα245-354 and U251 target cells with W6/32 led to the inhibition of the targeted cells lysis, whereas mouse IgG2, used as an isotype control, showed no effect. These results suggested that the HLA-A2/pIL-13Rα245-354-aAPCs–induced CTLs lysed the specific targets in a HLA class I–restricted manner.

Discussion

Malignant gliomas represent a significant class of CNS tumors derived from the glial lineage. Despite recent advances in traditional treatment options, the prognosis for these patients has not changed appreciably. The 5-year survival rate for patients harboring the most common class of gliomas, glioblastoma multiforme, is <2% (25). In an effort to improve the outcome of patients with respectable brain tumors, there have been attempts to give adjunctive therapies consisting of radiation with or without chemotherapy. Thus far, research over three decades has failed to provide definitive evidence of improved outcome (i.e., overall survival and disease-free survival) in patients. Clearly, modalities other than radiation and/or chemotherapy must be explored in an adjuvant setting. Immune-based treatments represent a promising new class of therapy designed to harness the immune system to specifically eradicate malignant cells. However, immunotherapy for tumors located in the CNS has generally not achieved the results seen for peripherally located tumors (26–29). These previous findings have historically been ascribed to the “immune privilege” of the CNS (30). In reality, however, the immune privilege of the brain is not absolute but was originally used to describe the observation that

Fig. 2. Detection of the HLA-A2/pIL-13Rα245-354-aAPCs–induced specific CTLs by ELISPOT assay. The CTLs induced by the HLA-A2/pIL-13Rα245-354-aAPCs were tested for ELISPOT assay. The effector cells were cocultured with HLA-A2/pIL-13Rα245-354-aAPCs or beads pulsed without HLA-A2/pIL-13Rα245-354 (as a control). After 24 h of incubation, the frequencies of individual antigen-specific IFN-γ–producing T cells were measured with an ELISPOT assay kit.

Fig. 3. Cytotoxic activity of HLA-A2/pIL-13Rα245-354-aAPCs–induced CTLs. The CTLs were induced with HLA-A2/pIL-13Rα245-354-aAPCs for 28 d. The cytotoxic activity of one individual’s CTLs is shown. The cytotoxic activity of the CTLs was assessed against U251 (HLA-A2+, IL-13Rα2+) and A172 (HLA-A2+, IL-13Rα2−) T2 cells pulsed without a peptide, T2pHIV (pHIV-pulsed T2 cells), T2pIL-13Rα245−354 (pIL-13Rα245−354−pulsed T2 cells), and K562 at various effector/target ratios (A). In addition, anti–HLA class I or a control antibody (mouse IgG2) was added to the T2pIL-13Rα245−354 and U251 targets, respectively, and incubated for 40 min at 4°C. After incubation, the target cells were mixed with effector cells for 51Cr release assay. The CTL assays were done at various effector/target ratios. Points, mean from triplicate wells; bars, SE. T2pIL-13Rα245−354 (B) and U251 (HLA-A2+, IL-13Rα2−; C) were used as target cells. Results show that the cytotoxicity of the HLA-A2/pIL-13Rα245-354-aAPCs–induced CTLs is pIL-13Rα245−354 specific and the CTLs lysed the specific targets in a HLA class I–restricted manner.
tissue and tumor grafts survived better in the CNS than in other peripheral sites (31). Effective anti-CNS tumor immune responses have been generated by immune-based treatments such as adoptive T-cell transfer (32–37), GAA-pulsed dendritic cells (3–5, 38), and cytokine-secreting glioblastomas or fibroblasts (39–41), lending further credence to the idea that the efficient induction of a cellular antitumor immune response can be targeted to antigens within the CNS.

Tumor-specific CD8-positive CTLs constitute the most important effector cells for antitumor responses (8). It has previously been shown that in vitro specific CTLs can be generated using peptide-pulsed autologous dendritic cells as APCs (42, 43). However, CTL expansion to clinically relevant amounts requires multiple leukopheroses to obtain enough autologous dendritic cells. Variability in both quantity and quality of obtained dendritic cells, which presumably relate to underlying diseases and the pretreatment of the patients, also significantly affects the viability of dendritic cell–based therapeutics. For these reasons, use of dendritic cells has been a limiting step in ex vivo expansion of T cells (18). Strategy of aAPC offers a promising way to overcome the disadvantages of natural APCs. CTLs generated by artificial stimulation protocols can kill both peptide-loaded and natural target cells (18, 44, 45). The presented data showed that CTLs specific for pIL-13Ra2345-354 could be induced in vitro with the aAPCs coating HLA-A2/pIL-13Ra2345-354 anti-CD28 antibody, and CD83 molecule to cell-sized latex beads. The HLA-A2+ PBMCs were induced to generate pIL-13Ra2345-354-specific CTLs in vitro by coculture with the HLA-A2/pIL-13Ra2345-354-aAPCs, and specificity of the aAPC-induced CTLs was confirmed by both their binding to HLA-A2/pIL-13Ra2345-354 tetramers and killing activity against HLA-A2/pIL-13Ra2345-354–bearing cells. Following this procedure, we found that the induced CTLs, derived from PBMCs in a HLA-A2+ healthy donor, could kill the HLA-A2+ pIL-13Ra2345-354 glioma cells when stimulated with HLA-A2/pIL-13Ra2345-354-aAPCs in vitro. The specific killing activity of CTL induced by the HLA-A2/pIL-13Ra2345-354-aAPCs against specific target cells T2pIL-13Ra2345-354 and U251 was much more effective than that of any other control group. The results indicated that the cytoxicity of the aAPCs-induced CTL is antigen specific (i.e., against the target cells bearing the corresponding HLA-A2/pIL-13Ra2345-354 complexes). In addition, it is possible to increase the percentage of antigen-specific CTL by sorting the cells after staining with tetramer and isolating the positive cells with magnetic beads loaded with anti-IFC antibody (Milenyi System). Many groups have applied this approach to obtain higher percentages of antigen-specific CTLs that could be further expanded with aAPC or beads coated with anti-CD3/CD28 mAb (46). Therefore, this technique can be extrapolated to expand enough T cells for clinical use.

For clinical studies, HLA tetramer–based aAPCs have several distinct advantages over cellular APCs including dendritic cells. One of these is ease of preparation, which is not required for sterile cell culture and cytokines, thereby reducing both the variability and expense associated with ex vivo expansion. The variability is particularly important when considering therapies for cancer because there have been reported defects in dendritic cells obtained from patients with malignancies (47). Another advantage is the good stability of aAPCs, unlike the biological variability and patient-to-patient variation when producing cellular APCs such as dendritic cells. In addition, aAPCs can present defined combinations of MHC alleles and peptides and be easily adapted using other MHC alleles and/or peptides, so that immunodominant or subdominant epitopes can be expanded preferentially. When cellular APCs are used, an array of MHC molecules is used and a broad but uncontrolled MHC restricted response is generated. The last advantage is the ability to control the combination of costimulatory complexes associated with aAPCs, unlike cellular APCs that may participate in the T-cell APC interaction in such a way that it promotes tolerance or anergy. For example, on T-cell activation, B7 binding to CTLA-4 instead of CD28 would limit T-cell expansion (48). It is convenient to prepare aAPCs coated with anti-CD28, which binds specifically to CD28, avoiding the binding of B7 to CTLA-4. In this study, we used a combination of anti-CD28 and CD83 for the generation of a costimulatory signal because CD83 molecule is important in (a) priming naïve CD8+ T cells; (b) driving their antigen-specific expansion; and (c) supporting their long-term survival and function when coupled to aAPCs (49). In contrast to other types of aAPCs previously engineered by others (50), there is no need for additional molecules like intercellular adhesion molecule-1 or lymphocyte function-associated antigen-3. The antigen-specific CTL can be successfully induced with the HLA-A2/pIL-13Ra2345-354-aAPCs in vitro. Further studies need to compare the efficiency of this approach with those of other techniques such as aAPCs with a combination of other costimulatory molecules.

The use of aAPC represents the state-of-the-art generation of antigen-specific CTLs for adoptive immunotherapy. Thus, HLA tetramer–based aAPCs coated with a HLA/peptide complex, anti-CD28 antibody, and CD83 molecule could provide a useful tool for the reproducible expansion of specific CTLs in vitro and significantly advance the field of adoptive immunotherapy. HLA-A2/pIL-13Ra2345-354-aAPCs could be used as a standardized, "off-the-shelf" reagent to enrich pIL-13Ra2345-354–specific CTLs for the treatment of patients with malignant gliomas.

**Acknowledgments**

We thank Drs. Wen-Hua Wu and Guo-An Chen in the Wuhan Blood Center for their kind assistance with this work.

**References**

5. Lau LM, Black KL, Prins RM, et al. Treatment of intracranial gliomas with bone marrow-derived dendritic...


Clinical Cancer Research

HLA Tetramer–Based Artificial Antigen-Presenting Cells Efficiently Stimulate CTLs Specific for Malignant Glioma

Xiaobing Jiang, Xiaoling Lu, Ruen Liu, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/13/24/7329

Cited articles
This article cites 50 articles, 15 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/13/24/7329.full.html#ref-list-1

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.