Identification of a Novel HLA-A*0201-restricted, Cytotoxic T Lymphocyte Epitope in a Human Glioma-associated Antigen, Interleukin 13 Receptor α2 Chain

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ABSTRACT

Purpose: Interleukin 13 receptor α2-chain (IL-13Rα2) has been reported to be abundantly and specifically overexpressed in glioblastoma multiforme. Here we report the identification of a CTL epitope derived from the IL-13Rα2.

Experimental Design: Mature dendritic cells (DCs) were pulsed with each of the synthetic peptides that were designed, based on a binding affinity-based prediction and a proteosomal cleavage site prediction system, and used to stimulate autologous CD8+ T cells from an HLA-A2+ healthy donor. After four to six cycles of restimulation, the immunoreactivity of the T cells was analyzed for specific IFN-γ production and CTL reactivity.

Results: Of the five peptides tested, IL-13Rα245–354 (WLPFGFILL) induced a CD8+ T-cell line that specifically produced IFN-γ in response to HLA-A2+ T2 cells pulsed with the relevant peptide and lysed these cells. Peptide titration assays demonstrated that half-maximal lysis of IL-13Rα245–354 peptide-reactive CD8+ T cells required peptide loading concentration of ~5 nM. Perhaps most importantly, this CD8+ T-cell line also displayed lytic activity against the HLA-A2+ human glioma cell lines that express IL-13Rα2.

Conclusions: This novel CTL epitope may therefore serve as an attractive component of peptide-based vaccines to treat glioma and as a surrogate marker of T-cell immune responses in patients before and after therapy.

INTRODUCTION

Although cellular mechanisms underlying the “immunologically privileged” status of the CNS and CNS tumors have been increasingly well-characterized during the past decade, it has also been becoming clearer that this “privileged” status is not absolute, and various new approaches of immunotherapy against CNS tumors have been suggested (1). Recent data from us (2, 3) and others (4) demonstrate that vaccinations with cytokine-gene-modified peripheral tumor cells can elicit therapeutic immune responses against intracranial gliomas. On the basis of our preclinical studies using IL-4-transfected glioma cells as vaccines (2, 3), we have begun Phase I clinical trials to test this strategy (5, 6). With regard to the concern of inducing an autoimmune reaction against normal brain components, no evidence of allergic encephalomyelitis was noted clinically or pathologically in 20 patients peripherally immunized with allogeneic glioma cells (7). A more practical limitation on the use of gene-modified whole cell brain tumor vaccines, however, is the considerable ex vivo manipulation of fresh glioma explants that is required to generate clinical grade vaccines. Tumor-specific, antigen-based vaccines, in contrast, would eliminate the potential risk of autoimmune encephalitis and be easily formulated using synthetic peptides and DCs generated from peripheral blood (8). We have reported preclinical efficacy of vaccinations using DCs loaded with synthetic peptides against intracranial tumors (9), and clinical efficacy of vaccinations with CTL epitopes has been demonstrated in other forms of cancers (8, 10). The development of glioma-specific, antigen-based vaccine therapy has awaits identification of glioma-specific, antigen-derived CTL epitopes. It has been well-documented that IL-13Rα2 is a glioma-associated antigen that has a cancer-testes antigen-type expression profile (11, 12). In this study, we assessed whether specific T-cell responses could be induced by IL-13Rα2-derived antigens in HLA-A*0201+ CD8+ T cells. For prediction of HLA-A*0201-restricted epitopes, we used a binding affinity-based prediction, BioInformatics & Molecular Analysis Section, and a proteosomal cleavage site prediction system. Our results indicate that we have isolated a novel HLA-A*0201-restricted CTL epitope in a glioma-associated antigen, IL-13Rα2.

3 The abbreviations used are: CNS, central nervous system; IL, interleukin; rhIL, recombinant human II; IL-13R, IL-13 receptor; PBMC, peripheral blood mononuclear cell; DC, dendritic cell; FBS, fetal bovine serum; RT-PCR, reverse transcription-PCR; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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MATERIALS AND METHODS

Cells and Cell Culture. Healthy volunteers were genetically typed as being HLA-A*0201™ at the Histocompatibility Center in Children’s Hospital (Pittsburgh, PA; University of Pittsburgh). PBMCs from an HLA-A*0201™ healthy donor were isolated using lymphocyte separation medium gradient (Ref. 13; Organon-Teknika, Durham, NC). The HLA-A*0201™-transfected, transporter associated with antigen processing-deficient (T x B) cell hybrid T2 cell line (T2.A2; provided by Dr. Janice Blum, Indiana University, Indianapolis, IN) was maintained in RPMI 1640 (Life Technologies, Inc., Grand Island, NY) supplemented with 10% FBS (Life Technologies, Inc.), 100 IU/ml penicillin, 100 μg/ml streptomycin, and 10 m M L-glutamine (all reagents from Life Technologies, Inc.).

The T98G and A172 glioma cell lines were purchased from the American Type Culture Collections (Manassas, VA). The U251 and SNB19 glioma cell lines were kindly provided by Drs. M. R. Jadus (University of Pittsburgh, Pittsburgh, PA; Ref. 14), respectively. These glioma cell lines uniformly express HLA-A*0201 and were cultured in DMEM supplemented with 10% FBS, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 10 m M L-glutamine (all reagents from Life Technologies, Inc.).

Selection and Synthesis of Candidate HLA-A*0201-binding Peptides Derived from IL-13Rα2. The protein sequences of IL-13Rα2 was obtained from GenBank and analyzed for HLA-A*0201 binding motifs using BioInformatics & Molecular Analysis Section and a proteosomal cleavage site prediction system (15), respectively. Peptide sequences that were given high binding scores and predicted proteosomal cleavage sites at the ends of the sequences were chosen. Peptides were synthesized by FMOC chemistry in the University of Pittsburgh Cancer Institute Peptide Synthesis Facility and were given high binding scores and predicted proteosomal cleavage sites. These glioma cell lines uniformly express HLA-A*0201 and were cultured in DMEM supplemented with 10% FBS, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 10 m M L-glutamine (all reagents from Life Technologies, Inc.).

RNA Isolation and RT-PCR Analysis of Expression of IL-13Rα2. For RT-PCR analysis, reverse transcription was performed using total cellular RNA extracted by the guani- diminium thiocyanate/cesium chloride method (16) in the presence of oligo dT and SuperScript II reverse transcriptase (Life Technologies, Inc., Gaithersburg, MD) according to the manufacturer’s instructions. The primers used for amplification of human IL-13Rα2 and GAPDH were as follows: IL-13Rα2 (S), 5’TGGTACAGAATGCTGCTGC-3’; (AS), 5’TCTGCCCAGGAACCTTGCC-3’; and GAPDH (S), 5’ACAGTCCGCGATCTTTCTC-3’; (AS), 5’TGTATTTGTGAGGGATTCTCG-3’.

RESULTS AND DISCUSSION

Induction of a T-Cell Line That Specifically Recognized Peptide IL-13Rα2345-354 Presented on HLA-A*0201™. On the basis of the algorithms, we synthesized the following five peptides: FILILVFV (350–358), YLQWQQPPLSL (50–59), WLPFGFILLI (345–354), WQCTNGSEV (111–119), and VIFVTL (355–363). DCs derived from a healthy HLA-A*0201™ donor were incubated with each of the five peptides (10 μg/ml) and used to stimulate autologous CD8+ T cells. The individual responder cell cultures were then restimulated on a weekly basis with autologous DCs or PBMCs loaded with the corresponding peptide used in the primary stimulation. Among the five peptides tested, four cycles of stimulation with IL-13Rα2345-354 resulted in continuous growth of responder T cells (data not shown). Specific immunoreactivity of the induced T-cell line was first tested with T2.A2 cells loaded with the relevant peptides, irrelevant peptides, or no peptides. As demonstrated in Fig. 1A, the T-cell line produced ~400 pg/ml of IFN-γ in response to T2.A2 cells pulsed with IL-13Rα2345-354, whereas it produced <50 pg/ml in response to T2.A2 cells alone.
SD.

bars,

experiment of three performed with similar results are depicted; production of IFN-

H9253
tide for 24 h. Supernatant samples were then harvested and analyzed for cultures; chromium release was measured. Data represent the average of triplicate

CTLs were then added at an E:T ratio of 10:1. After 4 h incubation,

GFILI) by the induced CD8

cells efficiently lysed T2 target cells pulsed with the relevant

were tested for cytotoxic activity. As shown in Fig. 1

B,

F

with the responder T cells at the indicated ratios for 4 h. Data shown

/H9262

g/ml IL-13R

323

with autologous DCs pulsed with the IL-

355

HLA-A*0201

A2 cells pulsed with the

in vitro

2345

T cells from an HLA-A*0201

354 peptide for 1 h and then labeled with

10 3 labeled target cells were then incubated

E/T Ratio

% Specific Lysis

0.01 1 10 100 1000

Fig. 1 Stimulation of CD8

T cells from a HLA-A*0201

healthy donor with IL-13Rx

peptide (WLPFGFILI) induced a CTL line that specifically recognized and lysed T2.A2 cells pulsed with the relevant peptide. CD8

T cells from an HLA-A*0201

healthy donor were stimulated in vitro with autologous DCs pulsed with the IL-

2345

peptide. A, aliquots of 1000 stimulated T cells were incubated in the presence of 5 x 10^5/well T2 cells pulsed with 10 μg/ml IL-13Rx

an irrelevant IL-13Rx

peptide, or without peptide for 24 h. Supernatant samples were then harvested and analyzed for production of IFN-γ by a specific ELISA. Data from one representative experiment of three performed with similar results are depicted; bars, SD. B, lysis of T2 cells pulsed with IL-13Rx

peptide (WLPFGFILI) by the induced CD8

T cells. T2.A2 cells were preincubated with 10 μg/ml IL-13Rx

peptide for 1 h and then labeled with

51Cr for 1 h. Aliquots of 5 x 10^3 labeled target cells were then incubated with the responder T cells at the indicated ratios for 4 h. Data shown is representative of three experiments with similar results. ○, T2 cells pulsed with IL-13Rx

peptide; □, T2 cells without peptides. Bars, SD. C, peptide titration of the CTL line specific for IL-13Rx

peptide. 51Cr-labeled T2 target cells were then incubated with the T2.A2 cells pulsed with various concentrations of IL-13Rx

peptide. The T8.IL-13R CTLs were then added at an E:T ratio of 10:1. After 4 h of incubation, chromium release was measured. Data represent the average of triplicate cultures; bars, SD.

or T2.A2 cells pulsed with the irrelevant peptide, IL-

355-364

indicating that the response was peptide specific. After two additional cycles of stimulation, the responder cells were tested for cytotoxic activity. As shown in Fig. 1B, the T cells efficiently lysed T2 target cells pulsed with the relevant peptide, whereas only low background lysis was observed in the absence of the peptide. These results demonstrated that the CTL line induced with IL-13Rx

recognized the relevant antigen-peptide specifically and was able to lyse cells presenting the peptide. The CTL line was thereby designated as T8.IL-13R.

Subsequently, the minimum stimulatory concentration was determined using the T8.IL-13R CTL line and T2.A2 cells loaded with various concentrations of the IL-13Rx

peptide. T2.A2 cells were pulsed with increasing concentrations of the peptide, and the lysis by T8.IL-13R was determined (Fig. 1C). Peptide titration demonstrated that half-maximal lysis by this T-cell line was obtained at IL-13Rx

concentrations between 10 and 100 nm. These results indicate that the T8.IL-13R line recognized IL-13Rx

with a sensitivity comparable with several known HLA-binding epitopes from nonmutated peptides, such as those derived from melanoma antigens (17, 18).

The T8.IL-13R Line Specifically Lysed HLA-A*0201

Glioma Cells That Express IL-13Rx

More importantly, we examined whether T8.IL-13R cells were able to recognize and lyse HLA-A*0201

human glioma cells that endogenously expressed and presented IL-13Rx

derived epitopes. Human glioma cell lines U251, SNB19, and T98G were determined to express HLA-A*0201 by a flow cytometric analyses, but human glioma cell line A172 did not express HLA-A*0201 (data not shown). Fig. 2 demonstrates the expression of IL-13Rx2 in these glioma cell lines as determined by semiquantitative RT-PCR. The U251, SNB19, and A172 cell lines expressed a high level message for IL-13Ro2, whereas the T98G cell line expressed a low level of message, and the IL-13Ro2-specific message was completely absent in PBMCs.

The lytic ability of T8.IL-13R against the HLA-A*0201

IL-13Ro2+ glioma cells was examined using 4-h 51Cr-release assays. As illustrated in Fig. 3, the U251 and SNB19 cell lines were highly susceptible to lysis by the CTL line, whereas lower, but significant, lysis was also observed using T98G cells. On the other hand, the HLA-A*0201

IL-13Ro2+ glioma cell line, A172, was not susceptible to lysis by the CTL line, suggesting that the CTL reaction is HLA-A*0201 restricted. To determine the specificity of the lytic activity, cold target competition experiments were performed by the addition of nonradiolabeled
T2.A2 cells pulsed with IL-13Ra2345–354 peptide in the 4-h 51Cr-release assay. The lytic activity of the CTL line against these glioma cell lines was almost completely inhibited by the addition of the cold target, demonstrating that the lytic ability of the CTL line raised against IL-13Ra2 was specific for the epitope IL-13Ra2345–354.

These data indicated that the CTL line raised against IL-13Ra2345–354 peptide was capable of recognizing and lysing HLA-A*0201+ glioma cell lines that endogenously expressed IL-13Ra2, suggesting that the IL-13Ra2345–354 peptide might be useful for inducing anti-glioma immunoreactivity.

Although information based on RT-PCR is only semiquantitative, U251 and SNB19 cells appeared to express a higher level of message for IL-13Ra2. Accordingly, flow cytometric analyses by Bernard et al. (19) have demonstrated high-level expression of IL-13Ra2 protein on the surface of U251 and SNB19 cells, whereas in the same study, T98G cells did not express a detectable level of IL-13Ra2. In another study (19), low levels of expression of IL-13Ra2 on T98G cells was described. In our study, susceptibility of U251, SNB19, and T98G cells against the T8.II.13 CTLs appears to be correlated with the expression level of IL-13Ra2; however, other factors, such as expression level of functional HLA-A*0201, must also be taken into consideration.

**Future Applications of the Novel Epitope IL-13Ra2345–354**

IL-13Ra2 has been reported by a significant proportion of malignant gliomas (11), and approximately 40–50% of Caucasians and Asians express the HLA-A*0201 allele (20). This novel CTL epitope may therefore serve as an attractive component of peptide-based vaccines to treat glioma and as a surrogate marker of T-cell immune responses in patients before and after therapy.

We also recognize that identification and subsequent implementation of one T-cell epitope as a therapeutic target may not be sufficient to improve the dismal prognosis of patients with malignant gliomas because of the marked heterogeneity of the disease. However, our efficient algorithms used for epitope prediction and in vitro analysis may soon lead to the identification of additional T-cell epitopes for glioma-derived antigens.

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**REFERENCES**


Article on Interleukin 13 Receptor α2 Chain

In the article on interleukin 13 receptor α2 chain in the September 2002 issue of Clinical Cancer Research, the amino acid positions for the IL-13Rα2 peptide were incorrectly stated. The actual positions are 345–353.

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