Induction of Adipophilin-Specific Cytotoxic T Lymphocytes Using a Novel HLA-A2-Binding Peptide That Mediates Tumor Cell Lysis

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ABSTRACT

Identification of tumor-associated antigens and advances in tumor immunology resulted in the development of vaccination strategies to treat patients with malignant diseases. Using a novel approach that combines DNA chip analysis of tumor samples with isolation of peptides on the surface of tumor cells, a HLA-A*0201-binding peptide derived from the adipophilin protein was identified. Adipophilin is involved in lipid storage and was thought to be expressed only in adipocytes, but it can be found in other cell types such as macrophages or tumor cells. In the present study, we analyzed the possible use of this peptide as a T-cell epitope presented by malignant cells. To accomplish this, we induced CTL responses using this HLA-A*0201-binding peptide. The in vitro-induced CTLs efficiently lysed cells pulsed with the adipophilin peptide and HLA-matched tumor cell lines in an antigen-specific and HLA-restricted manner. Finally, the induced CTLs recognized autologous dendritic cells (DCs) pulsed with the antigenic peptide or transfected with tumor RNA purified from an adipophilin-expressing tumor cell line. To further analyze the possible use of this peptide in immunotherapies of human malignancies, we induced adipophilin-specific CTLs using peripheral blood mononuclear cells and DCs from HLA-A*0201-positive patients with chronic lymphatic leukemia and plasma cell leukemia. The in vitro-generated CTLs recognized autologous chronic lymphatic leukemia cells and malignant plasma cells, whereas they spared nonmalignant resting or activated B and T lymphocytes, monocytes, or DCs. Our results demonstrate that this peptide might represent an interesting candidate for the development of cancer vaccines designed to target adipophilin-derived epitopes in a wide range of malignancies.

INTRODUCTION

The discovery of tumor-associated antigens and recent advances in the identification of T-cell epitopes using expression cloning, SEREX technology, or the “reverse immunology” approach opened new perspectives in the treatment of malignant diseases (1). Several Phase I/II studies have demonstrated that cancer vaccines designed to target epitopes derived from these tumor-associated antigens by CTLs can be successfully applied in several types of malignancies such as renal cell carcinoma (RCC), melanoma, prostate and breast cancer, or non-Hodgkin’s lymphoma, resulting in immunological and clinical responses (2–8). However, the application of these immunotherapeutic approaches is restricted by the limited number of known tumor antigens and defined T-cell epitopes, by patients’ HLA type, or by availability of tumor tissue.

DNA microarray analysis of tumor samples in comparison with normal tissue identified antigens. Combining gene expression profiling with analysis of MHC ligands by mass spectrometry, a HLA-A*0201-presented peptide derived from the adipophilin protein was recently described in a RCC (9). In the same study, it was demonstrated that in line with another previous report, adipophilin, a protein involved in lipid homeostasis of adipocytes and macrophages, is selectively overexpressed in some RCCs but only marginally found in nonmalignant tissues (10–14).

We analyzed the possible function of this peptide as a T-cell epitope and its presentation by malignant cells using antigen-specific CTLs that were generated by in vitro priming with monocyte-derived dendritic cells (DCs) as antigen-presenting cells. We show here that the CTLs generated from several healthy donors elicited an antigen-specific and HLA-A*0201-restricted cytolytic activity against tumor cells endogenously expressing the adipophilin protein including RCCs, breast cancer, melanoma, multiple myeloma cells, and primary autologous chronic lymphatic leukemia (CLL) cells or cells from plasma cell leukemia.

MATERIALS AND METHODS

Tumor Cell Lines. The following tumor cell lines were used in experiments: MCF-7 (breast cancer, adipophilin positive, HLA-A*0201 positive, purchased from American Type Culture Collection); A498, MZ1774, and MZ1257 (kindly provided by Prof. A. Knuth, Frankfurt, Germany); RCC cell lines, adipophilin positive, HLA-A*0201 positive; U266 (multiple myeloma, adipophilin positive, HLA-A*0201 positive); HCT116 (colon cancer, adipophilin positive, HLA-A*0201 positive); Mel1479 (malignant melanoma, adipophilin positive, HLA-A*0201 positive; kindly provided by Prof. G. Pawelec (Tübingen, Germany)); T2 [adipophilin positive, HLA-A*0201 positive, transporter associated with antigen presentation (TAP) deficient]; SK-OV-3 (ovarian cell line, adipophilin positive, HLA-A*0202 negative; kindly provided by O. J. Finn (Pittsburgh, PA)); Croft [EBV-immortalized B-cell line, adipophilin positive, HLA-A*0201 positive; kindly provided by (O. J. Finn)]. Tumor cell lines were grown in RPMI 1640 medium with glutamin, supplemented with 10% heat-inactivated FCS, 50 μM 2-mercaptoethanol, and antibiotics (Invitrogen, Karlsruhe, Germany). B cells from a patient with CLL and plasma cells from a patient with plasma cell leukemia were grown in RPMI medium for 24 h before they were used as target cells in a standard 51Cr release assay. K562 (chronic myelogogenous leukemia cell line) cells were used to determine the natural killer cell activity.

Cell Isolation and Generation of DCs from Adherent Peripheral Blood Mononuclear Cells (PBMCs). Generation of DCs from peripheral blood mononuclear cells was performed as described previously (15–17). In brief, PBMCs were isolated by ficoll/Paque (Biochrom, Berlin, Germany) density gradient centrifugation of blood obtained from buffy coat preparations of healthy donors from the blood bank of the University of Tübingen. Cells were seeded (1 × 10^7 cells/well) into 6-well plates (BD Falcon, Heidelberg, Germany) in RPMI 1640 medium. After 2 h of incubation at 37°C and 5% CO_2, nonadherent cells were removed, and the adherent blood monocytes were cultured in RPMI medium supplemented with the following cytokines: human recombinant granulocyte macrophage colony-stimulating factor (Leukomax; Novartis; 100 ng/ml); interleukin 4 (R&D Systems, Wiesbaden, Germany; 20 ng/ml); and tumor necrosis factor α (R&D Systems; 10 ng/ml). The phenotype of DCs was analyzed by flow cytometry after 7 days of culture (data not shown).

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B and T lymphocytes as well as monocytes were isolated from peripheral blood using magnetic cell sorting technology. The nonmalignant B cells were further purified by depletion of CD5+ B lymphocytes. Activation of B and T cells was performed as described recently (18). Monocytes were grown in medium supplemented with granulocyte macrophage colony-stimulating factor overnight before they were used as targets.

Reverse Transcription (RT)-PCR. RT-PCR was performed with some modifications, as described recently (15). Total RNA was isolated from cell lysates using Qiagen RNeasy Mini anion-exchange spin columns (Qiagen, Hilden, Germany) according to the instructions of the manufacturer. Up to 5 μg of total RNA were subjected to a 20-μl cDNA synthesis reaction (SuperScript First-Strand Synthesis System for RT-PCR; Invitrogen). Oligo(dT) was used as primer. One μl of cDNA was used in a 15-μl PCR amplification reaction. To control the integrity of the RNA, the efficiency of the cDNA synthesis, and the contamination with genomic DNA, 1 μl of cDNA was amplified by an intron-spanning primer pair for the β2-microglobulin gene. For the adipophilin and the β2-microglobulin cDNA, the PCR temperature profiles were as follows: 2-min pretreatment at 94°C, and 30 cycles at 94°C for 30 s, annealing at 59°C for 30 s, and 72°C for 60 s; with a final extension at 72°C for 7 min. Primer sequences were deduced from published cDNA sequences [β2-microglobulin, 5′-GGTTTCCTCCTCCTGCACAT-3′ and 5′-GATGCTGCTTACATGTCTCGA-3′; adipophilin (GenBank accession number NM_001122), 5′-GATCTGGTTCAGAAGCCAAGTTAT-3′ and 5′-CCTGATCTGGATGTTCTGTGGTA-3′]. Three to 5 μl of the RT-PCR reactions were electrophoresed through a 2% agarose gel and stained with ethidium bromide for visualization under UV light.

RNA Sources. Total RNA from human tissues (kidney, liver, adrenal gland, thymus, spleen, and bone marrow) was obtained commercially (Ambion, Houston, TX; Clontech, Heidelberg, Germany; Stratagene, Amsterdam, the Netherlands). The RNA from several individuals (between 2 and 62 individuals) was mixed in a way such that RNA from each individual was equally weighted. Quality and quantity were confirmed on the Agilent 2100 Bioanalyzer (Agilent, Waldbronn, Germany) using the RNA 6000 LabChip Kit (Agilent). Total RNA from DCs, leukocytes, CD3+ cells, and CD19+ cells was isolated from the blood of four volunteers using TRIZOL (Invitrogen) and mixed as described above.

Patient samples were obtained from the Department of Urology, University of Tübingen. The local ethical committee approved this study, and informed consent was obtained from the patients. All patients had histologically confirmed RCC. Fragments of normal and malignant renal tissue were dissected, shock-frozen, and ground by mortar and pestle under liquid nitrogen. Total RNA was prepared using TRIZOL according to the manufacturer’s protocol, followed by clean-up with RNeasy (Qiagen).

High-Density Oligonucleotide Microarray Analysis. Double-stranded DNA was synthesized from 5 μg of total RNA using SuperScript RTII (Invitrogen) and the primer (MWG Biotech, Ebersberg, Germany) as given by the Affymetrix manual. In vitro transcription using the BioArray High Yield RNA Transcription Labeling Kit (ENZO Diagnostics, Inc., Farmingdale, NY), fragmentation, hybridization on Affymetrix U133A GeneChips (Affymetrix, Santa Clara, CA), and staining with streptavidin-phyceroerythrin and biotinylated anti-streptavidin antibody (Molecular Probes, Leiden, the Netherlands) followed the manufacturer’s protocols (Affymetrix). The Affymetrix Gene-Array Scanner was used, and data were analyzed with the Microarray Analysis Suite 5.0 software. For normalization, 100 housekeeping genes provided by Array Scanner was used, and data were analyzed with the Microarray Analysis Suite 5.0 software.

Induction of Antigen-Specific CTL Response Using HLA-A*02-Restricted Synthetic Peptides. The HLA-A*0201-binding peptides derived from adipophilin (SSTTGG), survivin (ELTLGEFLKL; used as an irrelevant control), c-Met (YVPDVTSTI; used as an irrelevant control), and HIV (pol HIV-1 reverse transcriptase peptide; amino acids 476–484; ILKEPVGHV; used as an irrelevant control) were synthesized using standard F-moc chemistry on a peptide synthesizer (432A; Applied Biosystems, Weiterstadt, Germany) and analyzed by reverse-phase high-performance liquid chromatography and mass spectrometry. For CTL induction, 5 × 104 DCs were pulsed with 50 μg/ml synthetic adipophilin peptide for 2 h, washed, and incubated with 3 × 105 autologous PBMCs in RPMI medium. After 7 days of culture, cells were restimulated with autologous peptide-pulsed PBMCs. Two μg/ml human recombinant interleukin 2 (R&D Systems) was added on days 1, 3, and 5. The cytolytic activity of induced CTLs was analyzed on day 5 after the last restimulation in a standard 51Cr release assay (16, 17).

CTL Assay. The standard 51Cr release assay was performed as described previously (16, 17). Target cells were pulsed with 50 μg/ml peptide for 2 h and labeled with 51Cr-labeled sodium chromate in RPMI 1% for 1 h at 37°C and 5% CO₂. Cells (1 × 10⁶) were transferred to a well of a round-bottomed 96-well plate. Varying numbers of CTLs were added to give a final volume of 200 μl and incubated for 4 h at 37°C. At the end of the assay, supernatants (50 μl/well) were harvested and counted in a beta plate counter. The percentage of specific lysis was calculated as: 100 × (experimental release − spontaneous release)/maximal release − spontaneous release. Spontaneous and maximal releases were determined in the presence of either medium or 2% Triton X-100, respectively.

Antigen specificity of tumor cell lysis was further determined in a cold target inhibition assay (16, 17) by analyzing the capacity of peptide-pulsed unlabelled T cells to block lysis of tumor cells at a ratio of 20:1 (inhibitor: target ratio).

For antibody blocking experiments, cells were incubated for 30 min with 10 μg/ml of monoclonal antibody BB7.2 (IgG2b) recognizing HLA-A*02 or isotype antibody (ChromPure mouse IgG; Gianova, Hamburg, Germany; 0.2 μg/ml) before seeding in 96-well plates (16, 17).

IFN-γ ELISPOT Assay. Adipophilin-specific CTLs generated in vitro using autologous DCs pulsed with the adipophilin peptide (2 × 10⁵ cells/well) or PBMCs from a CLL patient, a patient with multiple myeloma, as well as PBMCs from healthy donors were incubated at a concentration 2 × 10⁵ cells/well in an autologous IFN-γ antibody monochlonal antibody 1-DIK; 10 μg/ml; Mabtech AB, Hamburg, Germany)-coated 96-well plate with autologous PBMCs pulsed for 1 h with the HLA-A2-binding peptides derived from the tumor antigens adipophilin, c-Met, or Her-2/neu (E75). For the detection of spots, a biotin-labeled antihuman IFN-γ antibody (monoclonal antibody 7-B6-1-Biotin; 2 μg/ml; Mabtech AB) was used. Spots were counted after 40–44 h of incubation using an automated ELISPOT reader (IMMUNOSPOT ANALYZER; CTL Analyzers LLC, Cleveland, OH).

Peptide Titration. Adipophilin-specific CTLs were generated as described above. For peptide titration, T2 cells were incubated with titrated amounts (10 to 10⁻⁷ μmol) of the adipophilin peptide. Corresponding specific CTLs were added to the target cells incubated with the cognate peptide at a ratio of 20:1.

PAGE and Western Blotting for Detection of Adipophilin Protein. Cells were lysed in buffer containing 1% Igepal, 50 mM HEPES (pH 7.5), 150 mM NaCl, 2 mM EDTA, 10% glycerin, 1 mM phenylmethylsulfonyl fluoride, and 2 μg/ml aprotinin. Protein concentration was determined using a BCA assay (Pierce, Rockford, IL). Twenty μg of total protein were separated on a 10% polyacrylamide gel, blotted on nitrocellulose membrane (Schleicher & Schuell), and probed with an adipophilin-specific antibody (guinea pig polyclonal antibody; GP40 mN1; Progen Biotechnik GmbH, Heidelberg, Germany) and an actin-specific antibody (goat polyclonal IgG sc-1616; Santa Cruz Biotechnology). Bands were visualized by enhanced chemiluminescence staining (Amersham Pharmacia, Freiburg, Germany; Ref. 18).

Electroporation of DCs with Enhanced Green Fluorescent Protein in Vitro Transcript or Whole Tumor-Derived RNA. Enhanced green fluorescent protein in vitro transcript was synthesized from the plasmid pSP64-Poly(A)-EGFPFI (generously provided by Y. F. I. Van Tendeloo, Antwerp, Belgium) as described previously (18, 19). Total RNA was isolated from cell lysates using Qiagen RNeasy Mini anion-exchange spin columns according to the protocol provided by the manufacturer. Quantity and purity of RNA were determined by UV spectrophotometry. RNA samples were routinely checked by formaldehyde/agarose gel electrophoresis for size and integrity and stored at −80°C in small aliquots.

Before electroporation on day 6, immature DCs were washed twice with serum-free X-VIVO 20 medium (BioWhittaker, Walkersville, MD) and resuspended to a final concentration of 2 × 10⁵ cells/ml. Subsequently, 200 μl of the cell suspension were mixed with 10 μg of total RNA and electroporated in a 4-mm cuvette using an Easyjet Plus unit (Peqlab, Erlangen, Germany). The
physical parameters were as follows: voltage of 300 V; capacitance of 150 μF; resistance of 1540 Ω; and pulse time of 231 ms. After electroporation, the cells were immediately transferred into RP10 medium and returned to the incubator.

RESULTS

Expression Analysis of Adipophilin. As demonstrated in Fig. 1, expression of adipophilin mRNA and protein could be detected in all tested human tumor cell lines. No adipophilin protein expression was found in human B and T lymphocytes, monocytes, and DCs.

We next performed high-density oligonucleotide microarray analysis to assess the expression of adipophilin mRNA in human RCCs compared to the corresponding normal renal tissues (Fig. 2). Overexpression of adipophilin was found in 8 of 12 tumor samples. We further compared the expression of adipophilin in the malignant tissue of a RCC patient with pooled RNAs derived from different donors and different nonmalignant tissues. In line with the results in Fig. 1, there was only marginal expression of adipophilin in normal tissues and cells from peripheral blood.

In contrast to previous reports, we could not find expression of adipophilin protein in peripheral blood monocytes, which might be due to the sensitivity of the antibody used.

Generation of Adipophilin-Specific CTLs Using Peptide-Pulsed DCs. To analyze the presentation of the adipophilin-derived HLA-A*0201-restricted peptide by human tumor cells, we induced specific CTLs in vitro using DCs derived from adherent PBMNCs of HLA-A*0201-positive healthy donors as antigen-presenting cells. The cytotoxicity of the in vitro-induced CTLs was assessed in a standard 51Cr release assay. The CTL lines obtained after several weekly restimulations demonstrated peptide-specific killing (Fig. 3, A and B). T cells only recognized T2 cells (TAP deficient) or autologous DCs coated with the cognate adipophilin peptide, whereas they did not lyse target cells pulsed with an irrelevant peptide, confirming the specificity of the cytolytic activity. To analyze the avidity of the induced CTL lines, T2 cells were incubated with titrated amounts of the synthetic peptide, and effector cells were added after a preincubation time of 1 h at an E:T ratio of 20:1. As shown in Fig. 3C, the adipophilin-specific CTLs lysed the target cells in an antigen concentration-dependent fashion with a sensitivity that ranged from 10 to 100 pm. To further characterize the effector functions of the adipophilin-specific CTLs, we analyzed the secretion of cytokines using an ELISPOT assay. Stimulation of the CTLs resulted in an antigen-specific production of IFN-γ as demonstrated in Fig. 3D.

The Identified Adipophilin Peptide Is Endogenously Processed and Mediates Tumor Cell Lysis. We next assessed the presentation of the endogenously synthesized adipophilin peptide in the context of the HLA-A*0201 molecule. In vitro-induced CTLs specific for the adipophilin peptide were tested for their ability to lyse human tumor cells expressing adipophilin. The adipophilin-positive and HLA-A*0201-expressing cell lines A498, MZ1257, and MZ1774 (RCC), MCF-7 (breast cancer), Mel1479 (malignant melanoma), and U266 (multiple myeloma) were used as target cells in a standard 51Cr release assay. The adipophilin-specific CTLs were able to efficiently recognize malignant cells expressing adipophilin in a HLA-A*0201-specific manner (Fig. 4, A and B).

Fig. 1. Expression of adipophilin in human tumor cell lines. Reverse transcription-PCR (A) using adipophilin-specific primers was performed to analyze the production of adipophilin mRNA. All cell lines tested were adipophilin positive. β2-Microglobulin (β2M) was used as a control for reverse transcription-PCR. A negative PCR control without cDNA was included (A). Western blot analysis (B) was performed using an adipophilin-recognizing polyclonal antibody. Adipophilin protein could be detected in tumor cell lines, whereas no expression was found in B cells, T cells, monocytes, and dendritic cells. Actin-specific polyclonal antibody was included as a control.

Fig. 2. mRNA expression of adipophilin in primary renal cell carcinoma and normal tissues. A, expression in tumors relative to autologous normal kidney, which was set as 1. B, expression is shown relative to pooled normal kidney, which was set as 1. All changes relative to kidney were significant according to the change algorithm. Error bars are given as 95% confidence intervals computed using the one-step Tukey’s Biweight method by taking a mean of the signal log ratios of probe pair intensities across the two arrays for each comparison.
There was no killing of the ovarian cancer cell line SK-OV-3 (HLA-A*02 negative, adipophilin positive) and only marginal recognition of the K562 cells, indicating that the cytotoxic activity was not natural killer cell mediated.

The antigen specificity and MHC restriction of the cytotoxic activity mediated by the peptide-induced CTL lines was further confirmed by using an HLA-A*02-specific monoclonal antibody and in cold target inhibition assays (Fig. 4C). The lysis of the A498 tumor cells could be blocked by incubation of target cells with the antibody specific for the HLA-A*02 molecule or the addition of cold (not labeled with 51Cr) T2 cells pulsed with the cognate peptide (Ad). T2 cells pulsed with an irrelevant peptide (Sv) were not lysed, either.

Adipophilin-Specific CTLs Can Lyse Autologous DCs Transfected with RNA Purified from the Adipophilin-Positive A498 Tumor Cell Line. Next, we analyzed the extent to which the CTLs are able to recognize target cells in an autologous setting and investigated the presentation of the identified adipophilin peptide on transfection of autologous HLA-A*0201-positive DCs with RNA from the adipophilin-expressing tumor cell line A498. As shown in Fig. 5, CTLs efficiently lysed autologous DCs pulsed with the cognate adipophilin peptide as well as DCs electroporated with the A498 RNA, indicating that the adipophilin peptide is processed and presented after transfection of DCs with RNA.

Adipophilin-Specific CTLs Recognize Autologous Malignant Cells. Using RT-PCR analysis, we found that primary malignant B cells from a patient with chronic lymphocytic leukemia express adipophilin (data not shown). The malignant B cells represented >90% of the peripheral blood lymphocytes and were cryopreserved before treatment of the patient. The patient was HLA-A*0201 positive and in remission after treatment with fludarabine. We generated adipophilin-specific CTLs from PBMCNs of the patient in remission and used autologous primary CLL cells as targets in a standard 51Cr release assay. The in vitro peptide-induced CTLs efficiently lysed autologous DCs from this patient that were pulsed with the cognate peptide as well as the autologous CLL cells, whereas they spared the nonmalignant B cells or the K562 cell line (Fig. 6). The specificity of the mediates cytotoxic T-cell response was further confirmed in a cold target inhibition assay using T2 cells pulsed with the cognate adipophilin or an irrelevant peptide (survivin).

Further RT-PCR analysis revealed that malignant cells from a HLA-A2-positive patient with plasma cell leukemia that developed from previously diagnosed multiple myeloma express adipophilin. The malignant plasma cells represented >90% of the blood population. Using PBMCNs derived from earlier time points, we were able to generate adipophilin-specific CTLs that lysed the autologous malignant cells, whereas they spared the nonmalignant B cells, T cells, monocytes, and DCs (Fig. 7).

In the next set of experiments we analyzed the frequency of adipophilin-reactive T cells in peripheral blood of healthy donors and two patients with malignant diseases (CLL and plasma cell leukemia). As shown in Fig. 8 IFN-γ-producing cells were detected in the peripheral blood of the patient with CLL as well as in the samples from donors 2 and 4.

DISCUSSION

Adipophilin, officially called adipose differentiation-related protein, was originally thought to be selectively expressed only in adipose tissue. However, adipophilin is expressed in various other tissues, including the ovary, testis, and skin. In addition, adipophilin is expressed in malignant cells, such as ovarian cancer cells and leukemia cells. The expression of adipophilin in malignant cells suggests that adipophilin may have a role in tumor development and progression. Our studies have shown that adipophilin-specific CTLs can efficiently lyse autologous malignant cells, indicating that adipophilin may be a target for immunotherapy in malignant diseases.
pocytes, but it can be found at the surface of lipid droplets in several other cell types such as monocytes and macrophages or human tumor cell lines (10–14). Comparative analysis using gene expression profiles of normal kidney and RCCs by differential display approach, quantitative RT-PCR, or DNA microarray technology revealed that adipophilin is highly overexpressed in some malignant RCC samples (9, 14). However, only low levels of adipophilin expression were detected in normal tissues, making this protein an interesting candidate of a cancer vaccine aimed to target this antigen.

Recently, a HLA-A*0201-binding peptide derived from the adipophilin protein was identified in tumors of RCC patients by applying an integrated functional genomics approach (9). This procedure combines comparative expression profiling of a tumor sample with the corresponding autologous normal tissue using DNA microarray technology to find genes overexpressed in the malignant cells with mass spectrometry to identify MHC class I ligands derived from these selectively expressed or overexpressed antigens.

We analyzed the possible use of this peptide as a T-cell epitope that can induce antigen-specific CTLs and mediate tumor cell lysis. To accomplish this, we used PBMCs from HLA-A*0201-positive donors in an in vitro immunization protocol. CTL induction was carried out by using monocyte-derived DCs pulsed with the adipophilin peptide as antigen-presenting cells. After several rounds of restimulations, cultures were tested for their lytic activity against target cells pulsed with the cognate adipophilin peptide or the irrelevant survivin peptide at an inhibitor:target ratio of 20:1.
adipophilin. Using RT-PCR and Western blot analysis, expression of adipophilin was found in several human tumor cell lines, indicating that this protein is expressed in a broad variety of human malignancies. The in vitro-induced peptide-specific CTLs were not only able to lyse target cells pulsed with the antigenic peptide but also recognized tumor cells endogenously expressing the adipophilin protein in an antigen-specific and HLA-A2-restricted manner, including RCC, malignant melanoma, breast cancer, and multiple myeloma cells. The specificity of the lytic activity was confirmed by the addition of a monoclonal antibody blocking the HLA-A2 molecules or by performing cold target inhibition assays.

To further analyze the specificity of the elicited CTL responses, we used autologous DCs that were either pulsed with the antigenic peptide or electroporated with RNA isolated from an adipophilin-expressing tumor cell line. The in vitro-generated CTLs efficiently lysed peptide-pulsed autologous DCs and DCs transfected with whole tumor RNA, demonstrating that the peptide used for CTL induction is also processed and presented on transfecion of DCs with whole tumor RNA.

Finally, we tested the ability of the identified adipophilin peptide to elicit CTL responses in patients with malignant diseases. CTL lines were generated from the PBMCs of a patient with CLL and from a patient with plasma cell leukemia by a first round of in vitro immunization with peptide-pulsed autologous DCs followed by two rounds of restimulations. The peptide-specific CTLs were used as effectors against the malignant cells. The expression of adipophilin in malignant cells that represented >90% of the peripheral blood lymphocytes in both patients was confirmed by RT-PCR (data not shown). The in vitro-induced CTLs efficiently lysed autologous leukemic cells in a MHC-restricted and antigen-specific manner but spared purified B and T cells as well as autologous DCs and monocytes from this patient. This demonstrates that adipophilin-specific CTLs can be generated in patients with malignant diseases and are able to recognize primary autologous tumor cells.

In conclusion, we have characterized a novel HLA-A*0201-restricted T-cell epitope derived from adipophilin, a protein involved in accumulation and storage of lipid. Adipophilin is expressed in a wide range of cultured tumor cell lines and overexpressed in some malignant tissues, such as RCC, but is only marginally expressed in nonmalignant organs. Our results demonstrate that adipophilin is a tumor rejection antigen expressed in many solid and hematopoietic malignancies and extend the number of possible CTL epitopes that can be used for the design of cancer vaccines.

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