Identification of C-Met Oncogene as a Broadly Expressed Tumor-Associated Antigen Recognized by Cytotoxic T-Lymphocytes

Kerstin Schag,1 Susanne M. Schmidt,1 Martin R. Müller,1 Toni Weinschenk,2 Silke Appel,1 Markus M. Weck,1 Frank Grünebach,1 Stefan Stevanovic,2 Hans-Georg Rammensee,2 and Peter Brossart1

1Department of Hematology, Oncology and Immunology, University of Tübingen, and 2Department of Immunology, Institute for Cell Biology, Tübingen, Germany

ABSTRACT

Purpose: C-Met proto-oncogene is a receptor tyrosine kinase that mediates the oncogenic activities of the hepatocyte growth factor. Using a DNA chip analysis of tumor samples from patients with renal cell carcinoma and sequencing of peptides bound to the HLA-A*0201 molecules on tumor cells a peptide derived from the c-Met protein was identified recently.

Experimental Design: We used this novel HLA-A*0201 peptide for the induction of specific CTLs to analyze the presentation of this epitope by malignant cells.

Results: The induced CTL efficiently lysed target cells pulsed with the cognate peptide, as well as HLA-A*0201-matched tumor cell lines in an antigen-specific and HLA-restricted manner. Furthermore, the induced c-Met-specific CTLs recognized autologous dendritic cells (DCs) pulsed with the peptide or transfected with whole-tumor mRNA purified from c-Met-expressing cell lines. We next induced c-Met-specific CTLs using peripheral blood mononuclear cells and DC from an HLA-A*0201-positive patient with plasma cell leukemia to determine the recognition of primary autologous malignant cells. These CTLs lyed malignant plasma cells while sparing nonmalignant B- and T-lymphocytes, monocytes, and DCs.

Conclusion: Our results demonstrate that c-Met oncogene is a novel tumor rejection antigen recognized by CTL and expressed on a broad variety of epithelial and hematopoietic malignant cells.

INTRODUCTION

C-Met encodes a heterodimeric transmembranous receptor with tyrosine kinase activity that is composed of an α chain that is disulfide-linked to a β subunit (1, 2). Both subunits are expressed on the surface; the heavy β subunit is responsible for the binding of the ligand, hepatocyte growth factor (HGF), and the α subunit contains an intracellular domain that mediates the activation of different signal transduction pathways. Met signaling is involved in organ regeneration, as demonstrated for liver and kidney, embryogenesis, hematopoiesis, muscle development, and in the regulation of migration and adhesion of normally activated B cells and monocytes. Furthermore, numerous studies indicated the involvement of c-Met overexpression in malignant transformation and invasiveness of malignant cells (3–12).

Using an integrated functional genomics approach that combines gene expression profiling with analysis of MHC ligands by mass spectrometry to identify genes and corresponding MHC ligands that are selectively expressed or overexpressed in malignant tissues an HLA-A*0201-presented peptide derived from the c-Met proto-oncogene could be identified (13). In the present study 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 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 c-Met protein including renal cell carcinomas, breast cancer, colon cancer, melanoma, and multiple myeloma cells. Furthermore, they lyed autologous DCs pulsed with the antigenic peptide or electroporated with RNA isolated from c-Met-expressing tumor cell lines and primary autologous malignant plasma cells while sparing nonmalignant B- and T-lymphocytes, monocytes, DCs, and bone marrow-derived or mobilized CD34+ hematopoietic cells. Our results demonstrate that c-Met oncogene is a tumor rejection antigen recognized by CTLs and expressed on a broad variety of epithelial and hematopoietic malignancies.

MATERIALS AND METHODS

Tumor Cell Lines. Tumor cell lines used in the experiments were grown in RPMI 1640 supplemented with 10% heat inactivated FCS and antibiotics. The following c-Met-expressing tumor cell lines were used in experiments: MCF-7 (breast cancer, HLA-A*0201+), purchased from American Type Culture Collection), A498 (renal cell carcinoma, HLA-A*0201+) and MZ1257 (renal cell carcinoma, HLA-A*0201+), kindly provided by Prof. Alexander Knuth, Frankfurt, Germany), U266 (multiple myeloma, HLA-A*0201+), HCT116 (colon cancer, HLA-A*0201+), Mel1479 (malignant melanoma, HLA-A*0201+), kindly provided by Prof. Graham Pawelec, University of Tübingen, Tübingen, Germany), and SK-
OV-3 (ovarian cell line, HLA-A*03+, kindly provided by O. J. Finn, Pittsburgh, PA). The c-Met-negative cell lines T2 (HLA-A*0201+, TAP-deficient) and Croft (EBV-immortalized B-cell line, kindly donated by O. J. Finn, Pittsburgh, PA, HLA-A*0201+) were used as controls. K562 cells were used to determine the natural killer-cell activity.

Cell Isolation and Generation of DC from Adherent Peripheral Blood Mononuclear Cells (PBMC). Generation of DCs from peripheral blood monocytes was performed as described previously (14). 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 × 10⁷ cells/3 ml/well) into six-well plates (BD Falcon, Heidelberg, Germany) in RPMI 1640 medium. After 2 h of incubation at 37°C and 5% CO₂, nonadherent cells were removed, and the adherent blood monocytes were cultured in RPMI 1640 medium supplemented with the following cytokines: human recombinant granulocyte macrophage colony-stimulating factor (Leukomax, Novartis; 100 ng/ml), interleukin 4 (R&D, Wiesbaden, Germany; 20 ng/ml), and tumor necrosis factor α (R&D; 10 ng/ml). The phenotype of DCs was analyzed by flow cytometry after 7 days of culture (data not shown).

Mobilized CD34+ hematopoietic progenitor cells, B- and T-lymphocytes, as well as monocytes were isolated from peripheral blood using magnetic cell sorting technology. Activation of B and T cells was performed as described recently (15). Monocytes were grown in RPMI 1640 medium with granulocyte macrophage colony-stimulating factor (Leukomax; Novartis; 100 ng/ml) overnight before being used as targets. Bone marrow cells and CD34+ progenitor cells were incubated for 24–48 h with stem cell factor (SCF; R&D; 100 ng/ml).

Reverse Transcription-PCR (RT-PCR). RT-PCR was performed with some modifications as described recently (14). Total RNA was isolated from cell lysates using Qiagen RNeasy “Mini” anion-exchange spin columns (Qiagen GmbH, Hilden, Germany) according to the instructions of the manufacturer and was subjected to a 20-μl cDNA synthesis reaction (Invitrogen, Karlsruhe, Germany). Oligodeoxynucleotidid acid was used as primer. One μl of cDNA was used for PCR amplification in a volume of 15 μl. To control the integrity of the RNA and the efficiency of the cDNA synthesis, 1 μl of cDNA was amplified by an intron-spanning primer pair for the β₂microglobulin gene. 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 for the c-Met and β₂microglobulin cDNA. Primer sequences were deduced from published cDNA sequences: β₂microglobulin: 5’ GGTTTCATCCTACCGCAT 3’ and 5’ GATGCTGTTACATGTCTCGA 3’, c-Met: 5’ ATGCAAGTGTTGATTTTGGT 3’ and 5’ ATGCGTCCCAATGGAGTGA 3’. Three to 5 μl of the RT-PCR reactions were electrophoresed through a 2.5% agarose gel and stained with ethidium bromide for visualization under UV light.

Induction of Antigen-Specific CTL Response Using HLA-A*0201-Restricted Synthetic Peptides. The HLA-A*0201 binding peptides derived from c-Met (YVDPVITSI, amino acids 654–662; Ref. 13), adipophilin (SVASTITGV; Ref. 15), survivin (ELTLGEFLKL; Ref. 16), and HIV (ILKEPVHG; pol HIV-1 reverse transcriptase peptide, amino acids 476–484) were synthesized using standard F-moc chemistry on a peptide synthesizer (432A; Applied Biosystems, Weiterstadt, Germany) and analyzed by reversed-phase high-performance liquid chromatography and mass spectrometry.

For CTL induction, 5 × 10⁵ DCs were pulsed with 50 μg/ml of the synthetic c-Met peptide for 2 h, washed, and incubated with 2.5 × 10⁴ autologous PBMC in RPMI 1640 medium. After 7 days of culture, cells were restimulated with autologous peptide-pulsed PBMC, and 2 ng/ml human recombinant interleukin 2 (R&D Systems) was added on days 1, 3, and 5. The cytolytic activity of induced CTL was analyzed on day 5 after the last restimulation in a standard 51Cr-release assay.

CTL Assay. The standard 51Cr release assay was performed as described (14). Target cells were pulsed with 50 μg/ml peptide for 2 h and labeled with [35S]sodium chromate in RPMI 1640 for 1 h at 37°C. Cells (10⁴) 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 β-plate counter. The percentage of specific lysis was calculated as: 100 × (experimental release – spontaneous release)/maximal release – spontaneous release). Spontaneous and maximal release were determined in the presence of either medium or 2% Triton X-100, respectively.

Antigen specificity of tumor cell lysis was additionally determined in a cold target inhibition assay (14) by analyzing the capacity of peptide-pulsed unlabeled T2 cells to block lysis of tumor cells at a ratio of 20:1 (inhibitor:target ratio).

IFN-γ Enzyme-Linked Immunospot Assay. C-Met-specific CTLs were generated in vitro using autologous DCs pulsed with the c-Met peptide. These CTLs were incubated at a concentration of 2 × 10⁵ cells/well in a 96-well plate coated with antihuman IFN-γ antibody (mAb 1-D1K, 10 μg/ml; Mabtech AB, Hamburg, Germany) together with autologous PBMCs pulsed for 1 h with the HLA-A2 binding peptides derived from the tumor antigens c-Met or adipophilin. For the detection of spots, a biotin-labeled antihuman IFN-γ antibody (Mab 7-B6-1-Biotin, 2 μg/ml; Mabtech AB) was used. Spots were counted after 40–44 h incubation using an automated enzyme-linked immunospot reader (IMMUNOSPOT ANALYZER; CTL Analyzers LLC, Cleveland, OH).

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

PAGE and Western Blotting. Cells were lysed in buffer containing 1% Igepal, 50 mM HEPES (pH 7.5), 150 mM NaCl, 2 mM EDTA, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, and 2 μg/ml aprotinin. Protein concentration of cell lysates were determined using a BCA assay (Pierce, Rockford, IL). Six to 30 μg of total protein were separated on a 7.5–9% polyacrylamide gel, blotted on nitrocellulose membrane, and probed with a c-Met-specific polyclonal antibody (h-Met, clone C-28, sc-161, polyclonal rabbit; Santa Cruz Biotechnology, Heidelberg, Germany). To ensure equal loading of the gel, the blots were reprobed using a polyclonal actin antibody (clone I-19; Santa Cruz Biotechnol-
Electroporation of DCs with Whole Tumor-Derived RNA. Total RNA was isolated from tumor cell lysates using RNeasy Maxi anion-exchange spin columns (Qiagen GmbH) according to the protocol for isolation of total RNA from animal cells provided by the manufacturer. Quantity and purity of RNA were determined by UV spectrophotometry. Before electroporation on day 6, immature DCs (grown in RPMI medium in the presence of interleukin 4 and granulocyte macrophage colony-stimulating factor) were washed twice with serum-free X-VIVO 20 medium (BioWhittaker, Apen, Germany) and resuspended to a final concentration of 2 × 10⁷ 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: voltage of 300 V, capacitance of 150 µF, resistance of 1540 Ω, and pulse time of 231 ms (17). After electroporation the cells were immediately transferred into RPMI 1640 medium and returned to the incubator.

RESULTS

Induction of c-Met-Specific CTLs Using Peptide-Pulsed DCs. C-Met proto-oncogene was shown to be overexpressed in a variety of malignant cells of epithelial and hematopoietic origin and to be involved in the malignant phenotype and invasiveness of these cells (3, 18–20). As demonstrated in Fig. 1, expression of c-Met mRNA and protein could be detected in several tested human tumor cell lines including malignant melanoma, renal cell carcinoma, and multiple myeloma.

Comparative analysis of gene expression profiling of a tumor sample derived from a patient with renal cell carcinoma and the corresponding autologous renal tissue was performed using DNA microarray technology followed by the characterization of MHC ligands present in the tumor by mass spectrometry. This resulted in the identification of an HLA-A*0201-presented peptide derived from the c-Met protein (YVDPVITSI, amino acids 654–662; Ref. 13). To analyze the presentation of this epitope by tumor cells and its recognition by CTLs we induced c-Met-specific CTLs in vitro using DCs derived from adherent PBMCs of HLA-A*0201-positive healthy donors. These monocyte-derived DCs were pulsed with the HLA-A*0201 binding c-Met peptide and used as antigen presenting cells for in vitro priming. Using this approach c-Met-specific CTLs were generated in 8 of 9 healthy donors.

The cytotoxicity of the induced CTL was analyzed in a standard ⁵¹Cr release assay using peptide-loaded T2 cells and autologous DCs as targets. As shown in Fig. 2, the CTL line obtained after several restimulations demonstrated antigen-specific killing. The T cells only recognized T2 cells (Fig. 2A) or DCs (Fig. 2B) coated with the cognate peptide, whereas they did not lyse target cells pulsed with irrelevant HLA-A*0201 binding peptides derived from survivin protein or HIV-1 reverse transcriptase 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 10:1. As shown in Fig. 2C, the c-Met-specific CTL lysed the target cells in an antigen concentration-dependent fashion with a sensitivity that ranged from 10 µM to 100 pM. To additionally characterize the effector functions of the c-Met-specific CTL we analyzed the secretion of cytokines using an enzyme-linked immunospot assay. The stimulation of the CTL resulted in an antigen-specific production of IFN-γ as demonstrated in Fig. 2D.

To determine the frequency of c-Met-reactive T cells we performed enzyme-linked immunospot assays using peripheral blood from healthy donors and 2 patients with malignant diseases (chronic lymphocytic leukemia and plasma cell leukemia). C-Met-specific T cells could be detected only in 2 of 10 healthy donors with 31 and 65 spots when 2 × 10⁵ PBMCs were used in the analysis (data not shown).

C-Met Peptide-Specific CTLs Efficiently Recognize Tumor Cells Endogenously Expressing the C-Met Proto-Oncogene. We next analyzed the ability of the in vitro induced CTL to lyse tumor cells that express the c-Met protein. We used the HLA-A*0201-positive cell lines HCT116 (colon cancer), A498, MZ1257 (renal cell carcinoma), MCF-7 (breast cancer), Mel1479 (malignant melanoma), and U266 (multiple myeloma) that express c-Met as targets in a standard ⁵¹Cr release assay. The EBV-transformed B-cell line Croft (HLA-A*0201+/c-Met−) and the ovarian cancer cell line SK-OV-3 (HLA-A3+/c-Met+) were included to determine the specificity and HLA-restriction of the CTL. As demonstrated in Fig. 3, A–D, the c-Met peptide-specific CTLs were able to efficiently lyse malignant cells expressing both HLA-A*0201 and c-Met. There...
Fig. 2  Induction of c-Met-specific CTL responses in vitro using peptide-pulsed dendritic cells (DC) as antigen presenting cells. DCs generated from adherent peripheral blood mononuclear cells in the presence of granulocyte macrophage colony-stimulating factor, interleukin 4, and tumor necrosis factor α were pulsed with the synthetic peptide derived from the c-Met protein and used for CTL induction. Cytotoxic activity of induced CTL was analyzed in a standard 51Cr release assay using T2 cells (A) or autologous DCs (B) pulsed with the cognate c-Met peptide (closed symbols) or an irrelevant peptide (HIV or survivin, open symbols) as targets. 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 10:1 (C). To analyze the antigen-specific secretion of IFN-γ by the in vitro-induced CTL an enzyme-linked immunospot assay was performed (D). The HLA-A2-binding adipophilin peptide was used as a negative control in this experiment.

Fig. 3  Antigen-specific lysis of human tumor cell lines endogenously expressing c-Met by c-Met-specific CTLs. Human HLA-A*0201+/c-Met+ colon cancer cell carcinoma cells HCT116, renal cell carcinoma (MZ1257 and A498), melanoma (Mel1479), breast cancer cell line MCF-7, multiple myeloma (U266) cell line, and the EBV-immortalized Croft cells (HLA-A*0201+/c-Met−) as well as the ovarian cancer cell line SK-OV-3 (HLA-A*0201/-c-Met+) were used as targets in a standard 51Cr release assay. K562 cells were included to determine the natural killer cell activity.
was no recognition of the ovarian cancer cells SK-OV-3 or Croft cells demonstrating that the presentation of c-Met peptide in the context of HLA-A*0201 molecules on the tumor cells is required for the efficient lysis of target cells and confirming the antigen specificity and MHC restriction of the CTLs. The in vitro-induced T cells did not recognize the K562 cells indicating that the cytotoxic activity was not natural killer-cell mediated.

To additionally verify the antigen specificity and MHC restriction of the in vitro-induced CTL lines we performed cold target inhibition assays (Fig. 4, A and B). The lysis of the target cells (U266, Fig. 4A, and A498, Fig. 4B) could be blocked in cold target inhibition assays. The addition of cold (not labeled with \(^{51} \text{Cr}\)) T2 cells pulsed with the cognate peptide reduced the lysis of tumor cells, whereas T2 cells pulsed with an irrelevant peptide showed no effect.

**C-Met-Specific CTLs Can Lyse Autologous DCs Transfected with Whole Tumor RNA.** To determine the cytotoxic activity of the CTLs in an autologous setting and test the presentation of c-Met-specific T-cell epitopes upon transfection with whole tumor RNA we used autologous DCs, generated from the same PBMCs that were used for CTL induction, as target cells. As shown in Fig. 5, CTLs efficiently lysed autologous DCs electroporated with the whole tumor RNA isolated from the c-Met-expressing A498 or MCF-7 tumor cell lines indicating that the identified c-Met peptide is processed and presented after transfection of DCs with RNA derived from c-Met-positive tumor cells.

In the next set of experiments we analyzed the cytolytic activity of the induced CTLs against human HLA-A*0201 matched bone marrow cells and mobilized CD34+ peripheral blood progenitor cells before and after incubation with SCF that was shown to induce differentiation of the progenitor cells and up-regulation of c-Met protein (10). As demonstrated in Fig. 6, c-Met-specific CTLs recognized autologous DCs pulsed with the antigenic peptide as well as HLA-A*0201-matched allogeneic malignant plasma cells expressing c-Met while ignoring HLA-A*0201-positive bone marrow cells or mobilized CD34+ peripheral blood progenitor cells that were stimulated with SCF or left untreated.

We next performed RT-PCR and Western blot analysis to determine the expression of c-Met in human mobilized CD34+ progenitor and bone marrow cells before and after exposure to SCF (Fig. 7). No expression of c-Met could be detected in CD34+ cells. Expression of c-Met was found to be very low in bone marrow cells compared with high expression in the renal cell carcinoma line (A498).

**C-Met-Specific CTLs Recognize Autologous Malignant Cells.** RT-PCR analysis revealed that malignant cells from a HLA-A*0201+ patient with plasma cell leukemia that developed from previously diagnosed multiple myeloma express c-Met (data not shown). The malignant plasma cells represented >90% of the blood population. Using PBMCs derived from earlier time points we were able to generate c-Met-specific CTLs that lysed the autologous malignant cells, whereas they spared the nonmalignant autologous B-cells, T-cells, monocytes and DCs (Fig. 8).
Deregulated control of the invasive growth phenotype by onco-
genically activated c-Met provoked by c-Met-activating mutations, c-Met amplification/overexpression, and the acquisition of HGF/c-Met autocrine loops confers invasive and metastatic properties to malignant cells. Notably, constitutive activation of c-Met in HGF-overexpressing transgenic mice promotes broad tumorigenesis (42, 43). Therefore, targeting of c-Met and/or c-Met oncogenic transduction pathways could represent a promising therapeutic option.

We show that c-Met-specific CTLs recognizing tumor cells in an antigen-specific and MHC-restricted manner can be induced in vitro suggesting that c-Met proto-oncogene is a novel tumor rejection antigen. We used the previously identified c-Met-derived HLA-A*0201-binding ligand for CTL induction and were able to demonstrate that this c-Met epitope is expressed on a broad spectrum of epithelial and hematological malignancies including renal cell carcinomas, breast cancer, colon cancer, malignant melanoma, and multiple myeloma indicating that this c-Met peptide is an interesting candidate for the development of a broadly applicable vaccination therapy. The specificity of the elicited CTL responses was confirmed in cold target inhibition assays. Furthermore, we performed the experiments in an autologous setting and used autologous DCs that were either pulsed with the cognate peptide or electroporated with RNA isolated from c-Met-expressing tumor cell lines as targets. The in vitro-induced c-Met peptide-specific CTL

**DISCUSSION**

Therapeutic vaccinations of patients with malignant diseases aim at stimulation of antitumor-directed immune responses, mainly CTLs, capable of recognizing and eliminating malignant cells. During the last years considerable efforts have been made to identify antigens specifically recognized by CTLs using reverse immunology, expression cloning, or Serex technology. However, with the exception of some tumor-associated antigens most of the identified T-cell epitopes are restricted to a limited set of malignancies (21–23).

Comparative expression profiling of a tumor and its corresponding autologous healthy tissue by DNA microarray technology allows the identification of antigens selectively expressed or overexpressed in malignant cells making these proteins suitable targets for immunotherapeutic approaches. Combination of this genetic analysis with mass spectrometric analysis of HLA ligands enables the characterization of antigenic peptides encoded by these antigens. Using this integrated functional genomics approach an HLA-A*0201-presented peptide derived from the c-Met proto-oncogene was identified (13).

C-Met is a heterodimeric tyrosine kinase receptor that mediates the multifunctional and potentially oncogenic activities of the HGF/scatter factor including promotion of cell growth, motility, survival, extracellular matrix dissolution, and angiogenesis (1–3). Binding of HGF to the receptor induces autophosphorylation of c-Met and activates downstream signaling events including the ras, phosphatidylinositol 3’-kinase, phospholipase Cγ, and mitogen-activated protein kinase-related pathways (4, 5, 24–27). The c-Met gene is expressed predominantly in epithelial cells and is overexpressed in several malignant tissues and cell lines (28–36). An increasing number of reports have shown that nonepithelial cells such as hematopoietic, neural, and skeletal cells respond to HGF and hematological malignancies like multiple myeloma, Hodgkin disease, leukemias, and lymphomas express the c-Met protein (37–41). Deregulated control of the invasive growth phenotype by onco-
genically activated c-Met provoked by c-Met-activating mutations, c-Met amplification/overexpression, and the acquisition of HGF/c-Met autocrine loops confers invasive and metastatic properties to malignant cells. Notably, constitutive activation of c-Met in HGF-overexpressing transgenic mice promotes broad tumorigenesis (42, 43). Therefore, targeting of c-Met and/or c-Met oncogenic transduction pathways could represent a promising therapeutic option.

We show that c-Met-specific CTLs recognizing tumor cells in an antigen-specific and MHC-restricted manner can be induced in vitro suggesting that c-Met proto-oncogene is a novel tumor rejection antigen. We used the previously identified c-Met-derived HLA-A*0201-binding ligand for CTL induction and were able to demonstrate that this c-Met epitope is expressed on a broad spectrum of epithelial and hematological malignancies including renal cell carcinomas, breast cancer, colon cancer, malignant melanoma, and multiple myeloma indicating that this c-Met peptide is an interesting candidate for the development of a broadly applicable vaccination therapy. The specificity of the elicited CTL responses was confirmed in cold target inhibition assays. Furthermore, we performed the experiments in an autologous setting and used autologous DCs that were either pulsed with the cognate peptide or electroporated with RNA isolated from c-Met-expressing tumor cell lines as targets. The in vitro-induced c-Met peptide-specific CTL
Induction of c-Met-Specific CTL

B the lysis is not natural killer cell mediated (c-Met-specific CTL do not lyse autologous monocytes and resting or activated B and T cells. K562 cells were included as a control, indicating that the lysis is not natural killer cell mediated (B).

efficiently lysed peptide-pulsed autologous DCs and DCs transfected with whole tumor RNA, thus demonstrating that the peptide used for CTL induction is also processed and presented upon transfection of DCs with whole tumor RNA and might, therefore, represent a very useful epitope in cancer vaccinations.

Using our in vitro priming approach with peptide-pulsed DCs we were able to generate c-Met-specific CTLs that lysed autologous malignant cells from a HLA-A*0201+ patient with plasma cell leukemia, whereas they spared the nonmalignant autologous B cells, T cells, monocytes, and DC.

Previous studies have shown that under normal conditions c-Met gene can be found in many epithelial tissues, and its expression can be induced by treatment with phorbol esters, serum, and in a paracrine fashion by mesenchymally derived HGF. HGF/scatter factor-Met signaling is required for normal development of liver, skeletal muscle, and placenta (37). In the hematopoietic system, HGF is produced by stromal bone marrow cells and together with other cytokines and growth factors induces proliferation and differentiation of a subset of c-Met-positive progenitor cells. The c-Met/HGF interaction plays an important role in the lymphoid microenvironment and induces adhesion as well as migration of normally activated B cells and monocytes (3–12).

These observations indicate that the c-Met/HGF network promotes pleiotropic effects in normal cells and is not a cancer-specific antigen, and caution is required when targeting this protein in clinical vaccination trials. However, we were not able to detect any significant recognition of nonmalignant B and T lymphocytes, DCs, monocytes, or hematopoietic progenitor cells by c-Met-specific CTLs in our in vitro assays.

As mentioned above it was demonstrated recently that bone marrow cells can up-regulate the expression of c-Met upon treatment with SCF. However, these cells were not recognized by the in vitro-induced c-Met-specific CTLs as shown in Fig. 6. PCR and Western blot analysis demonstrated low C-Met expression in bone marrow cells as compared with the A498 renal cell carcinoma cell line indicating that the level of antigen expression that is higher in malignant cells correlates with the ability of the in vitro-generated CTLs to recognize and lyse c-Met-presenting target cells.

In several clinical vaccination trials using DCs presenting tumor-associated antigens or adoptive transfer of tumor-reactive CTLs generated ex vivo, it was shown that these approaches can induce antitumor immunity in patients with malignant diseases (22, 23, 44–48). However, with the exception of some reports in malignant melanoma trials where induction of vitiligo was observed after vaccinations with DCs even when antigens that are expressed in normal tissues like MUC1 or Her-2/neu were applied there has thus far no evidence for the development of autoimmune reactions in these patients (49). In conclusion, in our study we describe the identification of a novel broadly expressed T-cell epitope derived from a proto-oncogene that is an interesting candidate to be applied in immunotherapies of human malignancies.

ACKNOWLEDGMENTS

We thank Bruni Schuster and Sylvia Stephan for excellent technical assistance. We thank Tina Wiens, Andreas M. Boehmler, and Hans-Joerg Buehring for providing CD34+ and bone marrow cells.

REFERENCES


5. Montesano R, Soriano JV, Malinda KM, Ponce ML, Bafico A, Kleinman HK, Bottaro DP, Aaronson SA. Differential effects of hepa-


