

A Point Mutation in the α -Actinin-4 Gene Generates an Antigenic Peptide Recognized by Autologous Cytolytic T Lymphocytes on a Human Lung Carcinoma¹

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ABSTRACT

We have identified an antigen recognized on a human large cell carcinoma by an autologous tumor-specific CTL clone that was derived from mononuclear cells infiltrating the primary tumor. The antigenic peptide is presented by HLA-A2 molecules and is encoded by the α -actinin-4 gene, which is expressed ubiquitously. In the tumor cells, a point mutation generates an amino-acid change that is essential for recognition by the CTLs. The mutation was not found in α -actinin-4 cDNA sequences from about 50 lung carcinoma cell lines, suggesting that it is unique to this patient. Although he did not receive chemotherapy or radiotherapy, the patient has been without evidence of tumor since the resection of the primary lesion in 1996. Using tetramers of soluble HLA-A2 molecules loaded with the mutated antigenic peptide, anti- α -actinin-4 CTLs could be derived from blood samples collected from the patient in 1998 and 2000. It is possible that these CTLs, recognizing a truly tumor-specific antigen, play a role in the clinical evolution of this lung cancer patient.

INTRODUCTION

A large number of antigens recognized by CTL derived from blood lymphocytes or TILs⁴ were identified over the last 10 years (1). Some of these antigens, such as those encoded by the *MAGE-A* genes, are strictly tumor-specific and shared by many tumors and, therefore, constitute promising targets for anticancer immunization. Antigenic peptide MAGE-A3.A1, encoded by gene *MAGE-A3* and presented to CTL on HLA-A1 molecules, has been used to vaccinate tumor-bearing metastatic melanoma patients. Tumor regressions were observed in a significant proportion (7 of 25) of the patients (2). Regressions of metastases were also observed in melanoma patients who received injections of autologous dendritic cells incubated with the MAGE-A3.A1 peptide and a recall antigen (3). Although the mechanism underlying these tumor regressions is still unclear, these results suggest that defined tumor-specific antigens recognized by CTL may be used in therapeutic vaccines, without significant toxicity.

The majority of tumor antigens have been identified with CTL clones that recognized autologous melanoma cells. Much less is

known about tumor-specific antigens recognized by CTL on lung tumors. Most of the latter belong to the categories of NSCLC or SCLC. NSCLC includes squamous carcinomas, adenocarcinomas, and large cell carcinomas and represents 80% of lung cancers. These tumors are often infiltrated by T cells, most of which are T-cell receptor α/β^+ , CD8⁺, CD28⁺ (4).⁵ The role and target antigens of these T cells are not known. NSCLC cell lines are difficult to establish, but a few of them could be used to stimulate autologous lymphocytes and derive tumor-specific CTLs (5–7). Two tumor-specific antigens were identified. One of them is encoded by HER2/neu, which is overexpressed in many tumors (8). The antigenic peptide, presented by HLA-A2 molecules, was initially identified with CTLs derived from TILs. The other antigenic peptide that was found to be recognized by CTLs on NSCLC is encoded by a mutated elongation factor 2 gene and presented by HLA-A68.2 molecules (9). Recently, two antigenic peptides presented by HLA-A24 molecules and reported to be recognized by tumor-specific CTLs were found to be encoded by the cyclophilin B gene, which is expressed ubiquitously (10), and peptides derived from an endoplasmic reticulum-resident protein were shown to be presented on HLA-A24 molecules to CTLs derived from TILs (11).

We have derived antitumor CTL clones by stimulating lymphocytes infiltrating the large cell carcinoma of a patient named Heu (4) with the autologous tumor cell line. In this study, we report on the identification of an antigen recognized by one of these CTL clones.

MATERIALS AND METHODS

Patient Characteristics and Clinical Course. Patient Heu, a 65-year old Caucasian man, presented in 1996 with an undifferentiated large cell carcinoma of the left lung. No other tumoral localizations were found, and the primary tumor (pT₂; N0) was resected. The tumor lesion was massively necrotic and infiltrated with T lymphocytes (4). The patient received no other treatment and has been without evidence of cancer since then. At the time of surgery and 1, 2.5, 3, and 4 years later, blood samples were collected, and mononuclear cells were isolated and frozen.

Derivation and Culture of CTL Clone Heu127. NSCLC cell line IGR-Heu was derived from a biopsy of the large cell lung carcinoma of patient Heu (HLA-A2, A68, B7, B35, C4, C7; Ref. 12). IGR-Heu cells were maintained in DMEM:Ham's F-12 medium supplemented with 10% FCS (Seromed, Berlin, Germany). CTL clone Heu127 was derived as described (4). Briefly, a fresh tumor sample from patient Heu was dissociated in DMEM medium containing 1 mM HEPES, 0.3 units/ml DNase, 0.5 units/ml collagenase, and 0.28 units/ml hyaluronidase (Life Technologies, Inc., Cergy Pontoise, France), and the resulting cell suspension was frozen. After thawing, viable TILs and tumor cells were isolated using Ficoll-Hypaque (Pharmacia Fine Chemicals, Uppsala, Sweden) density gradient centrifugation. The lymphocytes were seeded at 10⁴ cells/microwell and stimulated by the addition of irradiated (10,000 rads) autologous tumor cells (3.10³/well) and irradiated autologous EBV-transformed B cells (Heu-EBV, 4.10⁴/well) in RPMI 1640 medium supplemented with 10% human AB serum (Institut Jacques Boy, Reims, France) and rIL-2 (20 units/ml; Roussel-Uclaf, Romainville, France). Cells were fed every 3 days

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⁴ The abbreviations used are: TIL, tumor-infiltrating lymphocyte; NSCLC, non-small cell lung cancer; IL, interleukin; TNF, tumor necrosis factor; PBMC, peripheral blood mononuclear cell; HS, human serum; mAb, monoclonal antibody; poly(A), polyadenylate.

⁵ H. Echchakir, I. Vergnon, F., and Mami-Chouaib, unpublished data.

with medium and IL-2 and restimulated every other week with irradiated IGR-Heu cells and irradiated Heu-EBV cells. After 3 weeks, the resulting cell line was cloned by limiting dilution (0.5 cell/well) in 96-well V-shaped microtiter plates (Nunc, Roskilde, Denmark) in the presence of irradiated IGR-Heu (3.10^3 /well) and Heu-EBV (4.10^4 /well) cells, rIL-2 (100 units/ml), and 3% of conditioned medium from phytohemagglutinin-activated lymphocytes. CTL clone Heu127 was restimulated every other week with this same protocol.

Functional Assays. Lytic activity was measured with a conventional ^{51}Cr -release assay of 4 h using 1000 target cells/well. Inhibition of lysis by anti-HLA-A2 antibody MA2.1 (American Type Culture Collection) was tested by preincubating target cells for 2 h at 37°C with a saturating amount of ascitic fluid from mice inoculated with the hybridoma cells. TNF- α was detected by measuring the cytotoxicity of the culture medium on the TNF-sensitive WEHI-164c13 cells (13) with a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide colorimetric assay (14).

Construction and Screening of the cDNA Library. About 3 μg of poly(A)⁺ RNA extracted from IGR-Heu cells with the Fastrack kit (Invitrogen, Groningen, the Netherlands) was converted to cDNA with the Superscript Choice System (Life Technologies, Inc., Gaithersburg, MD) using the oligodeoxythymidylate primer 5'-ATAAGAATGCGCCGCTAACTA(T)₁₈VN ($V = \text{G, A, or C}; N = \text{G, A, T, or C}$), which contains a *NotI* site at its 5' end. The degenerate 3' end of the oligonucleotide favors annealing at the 5' end of the poly(A) tail of the mRNA molecules, reducing the proportion of cDNA clones containing long poly(A) sequences, which are difficult to sequence. The cDNA was ligated to *HindIII-EcoRI* adapters (Stratagene, La Jolla, CA), phosphorylated, digested with *NotI*, and ligated to plasmid pCEP4 (Invitrogen), which had been digested previously with *HindIII* and *NotI* and dephosphorylated. Recombinant plasmids were electroporated into *Escherichia coli* DH5 α and selected with ampicillin (50 $\mu\text{g}/\text{ml}$). The library was divided into pools of approximately 100 cDNA clones. Each pool was amplified, and plasmid DNA was extracted using the QIAprep8 plasmid kit (Qiagen, Hilden, Germany). Duplicate microcultures of 293-EBNA cells (Invitrogen), plated in flat-bottomed 96 microwells ($5-7 \times 10^4$ /well) 24 h before transfection, were cotransfected with about 100 ng of plasmid DNA of each pool of the cDNA library, 50 ng of expression vector pcDNA1/Amp (Invitrogen) containing an HLA-A*0201 cDNA clone, and 1.3 μl of LipofectAMINE reagent (Life Technologies, Inc.). After 24 h, CTL clone Heu127 (3000 cells/well) was added. After another 24 h, half of the medium was collected, and its TNF content measured with the WEHI-164c13 cells.

Sequence Analysis and Localization of the Antigenic Peptide. cDNA clone 57 was sequenced using dideoxy chain method in an ABI 310 automated DNA sequencer (Perkin-Elmer Applied Biosystems, Warrington, United Kingdom).⁶ Sequence alignments were performed with Geneworks software (Intelligenetics, Mountain View, CA). To identify the antigenic peptide, two cDNA fragments were amplified from cDNA clone 57 with PCR using forward primer OPC1059 (5'-AAGATGAGAGTGCACAAAATCAAC), which contains a Kozak ATG in frame with the longest open reading frame of the cDNA, and reverse primers OPC1061 (5'-GAGCCCTTCCTTGGCCGA) and OPC1062 (5'-GCGGTCTGGTGCGCA). PCR conditions were 3 min at 94°C followed by 30 cycles consisting of 1 min at 94°C , 2 min at 61°C (OPC1061) or 67°C (OPC1062), and 3 min at 72°C , followed by a final elongation step of 15 min at 72°C . PCR products were purified using QIAEX II agarose gel extraction kit (Qiagen) and cloned into expression plasmid pcDNA3.1 using the Eukaryotic TOPO TA Cloning Kit (Invitrogen). The constructs were cotransfected, using LipofectAMINE as above, with the HLA-A2 cDNA clone into 293-EBNA cells.

Screening for α -Actinin-4 Mutations. Total RNA extracted from 56 tumor cell lines (NSCLC, SCLC, melanoma, and thyroid) was converted to cDNA with the M-MLV reverse transcriptase (Boehringer Mannheim, Mannheim, Germany) using an oligodeoxythymidylate primer. The cDNA were used as templates for PCR amplification using primers OPC1216 (5'-ATGGGCGACTACATGGCC) and OPC1217 (5'-CGGTTGGCGGCAGTTTCA) for 30 cycles of 1 min at 94°C , 2 min at 63°C , and 2 min at 72°C . PCR products were purified using QIAquick PCR purification Kit (Qiagen) and sequenced.

Tetramer Analysis. An HLA-A*0201 cDNA clone was used as a template to amplify the sequence coding for the extracellular domains (amino acids 1–276 of the mature protein) of the HLA-A*0201 heavy chain with primers AIM8 (5'-AAGAAGGAGATATACCATGGGTCaCACagTATGgcTATTTTtTtACATCCGTGTCCCGG) and A2b (5'-ATGATGCAGGGATCCTTCGAAGATGTCGTTcAGACCACCACCCGGCTCCCATCTcAGGGTG). AIM8 contains several base changes (small letters) designed to optimize protein expression in *outl. E. coli* BL21(DE3)pLysS. The PCR product was digested with *NcoI* and *SfiI* and cloned into a vector derived from pET3D (Stratagene) and containing a BirA biotinylation site in frame with the 3' end of the HLA sequence. Recombinant HLA-A*0201 molecules were folded *in vitro* with $\beta 2$ -microglobulin (produced from pHN1- $\beta 2\text{m}$, kindly provided by P. Moss, Oxford University) and the mutated α -actinin-4 peptide, as described (15). Soluble complexes purified by gel filtration were biotinylated using the BirA enzyme (Avidity LCC, Denver, CO). Phycoerythrin-labeled tetramers were produced by mixing the biotinylated complexes with extravidin-phycoerythrin (Sigma Chemical Co., St. Louis, MO). For tetramer-staining experiments, PBMC ($1-2 \times 10^6$) were thawed, an aliquot was taken for staining, and the remaining cells were stimulated as follows. Cells were incubated over 1 h at ambient temperature with the mutated α -actinin-4 peptide (20 μM) in Iscove's medium containing 1% HS, washed, and cultured at about 10^6 cells/1 ml wells in Iscove's medium containing 10% HS, IL-2 (20 units/ml), IL-4 (10 ng/ml), and IL-7 (10 ng/ml). After 7 days, an aliquot of the responding cells was used for tetramer staining, and the remaining cells were restimulated and cultured as above. After an additional 7 days, the cells were collected, washed, and stained. Positive cells were sorted and seeded at 1 cell/well in round-bottomed microplates using a FACS-VANTAGE (Becton Dickinson, San Jose, CA). The lymphocytes were restimulated by the addition of irradiated allogeneic HLA-A2-matched EBV-B cells (2×10^4 /well), incubated over 1 h at ambient temperature with antigenic peptide (20 μM) and washed, 100 units/ml IL-2, 10 ng/ml IL-4, 10 ng/ml IL-7, and irradiated feeder cells consisting of allogeneic PBMC (8×10^4 /well). The same protocol of stimulation was applied after 1 week of culture, and 7–15 days later, clonal populations were labeled with tetramer. Some of them, chosen randomly, were used in a stimulation assay (production of TNF) with an HLA-A2 melanoma cell line (MZ-2-MEL cells transfected with an HLA-A2 construct) incubated with or without 1 nM of the mutated α -actinin-4 peptide. For the tetramer labeling, cells were resuspended in PBS + 1% HS and incubated for 15 min at ambient temperature with tetramer coupled to phycoerythrin. Antibodies (anti-CD3 coupled to FITC and anti-CD8 coupled to PerCP, Becton Dickinson) were then added for an additional 15 min at 4°C .

Assessment of Peptide/HLA-A*0201 Complex Stability. T2 cells (10^6 /ml) were incubated overnight at 37°C with 100 μM of each peptide in serum-free RPMI 1640 medium supplemented with 100 ng/ml of $\beta 2\text{m}$. Cells were then washed four times to remove free peptides, incubated with brefeldin A (10 $\mu\text{g}/\text{ml}$) for 1 h to block cell surface expression of newly synthesized HLA-A*0201 molecules, washed, and incubated at 37°C for 0, 2, 4, and 6 h. Subsequently, the cells were stained with mAb BB7.2 followed by FITC-conjugated goat-antimouse immunoglobulin mAb. For each time point, the expression of HLA-A*0201 induced by the peptide was calculated as mean fluorescence of cells preincubated with peptide – mean fluorescence of cells treated in similar conditions but in the absence of peptide.

RESULTS

A CTL Clone Recognizing Autologous Lung Tumor Cells. The NSCLC cell line IGR-Heu was derived from a tumor resected from patient Heu in April 1996. Mononuclear cells that infiltrated the tumor were isolated and stimulated on day 0 and 14 with irradiated IGR-Heu cells, irradiated autologous EBV-transformed B cells, and IL-2. On day 21, the responder lymphocytes were cloned by limiting dilution. A panel of tumor-specific CTL clones was obtained, which could be classified into three groups on the basis of the sequence of their T-cell receptor β chain (4). CTL clone Heu127 expressed a V $\beta 22$ -J $\beta 1.4$ rearrangement. It lysed the autologous tumor cells IGR-Heu but not autologous EBV-B cells or the NK-target cells K562 (Fig. 1A). Patient Heu was typed HLA-A2 by serology, and the recognition of IGR-Heu cells by CTL Heu127 was inhibited by the anti-HLA-A2 mAb MA2.1 (data not shown).

⁶ A computer search for sequence homology was performed using programs available at <http://www.ncbi.nlm.nih.gov/blast/blast.cgi>.

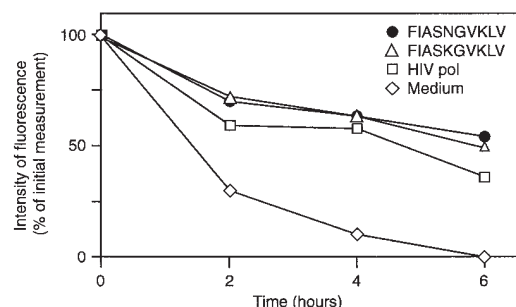


Fig. 3. Binding of peptides to HLA-A2 molecules. Peptides (100 μ M) were incubated overnight with T2 cells, which were then washed, treated over 1 h with brefeldin A, washed, and incubated at 37°C for the indicated times. The cells were then labeled with an anti-HLA-A2 mAb. The intensities of staining are expressed relative to those observed in the initial labeling experiment, which was performed immediately after the treatment with brefeldin A.

and April 2000. These PBMCs were stimulated with the antigenic peptide and IL-2, IL-4, and IL-7. After 1 week, CD8⁺ α -actinin-4-specific cells could be detected with frequencies of 0.7 and 3.6% of the CD8 cells in blood collected in 1998 and 2000, respectively (Fig. 5). After another restimulation with the antigenic peptide, the proportion of tetramer positive cells increased to 56% of the CD8 cells derived from the 1998 blood collection. To verify the specificity of the staining, the tetramer-positive cells were seeded at one cell/well and stimulated with irradiated HLA-A2 EBV-B cells incubated with the antigenic peptide, irradiated feeder cells, IL-2, IL-4, and IL-7. The CTL clones that were obtained proved to be specific for the mutated α -actinin-4 peptide, as shown in a TNF secretion assay (Fig. 5).

DISCUSSION

α -Actinins are proteins of about M_r 100,000 encoded by members of the spectrin gene superfamily. They contain an NH₂-terminal actin-binding domain, a central rod composed of four spectrin-like repeats (17), and a calmodulin-like domain at the COOH terminus. Organized as antiparallel homodimers, they form a rod-shaped complex with an actin-binding domain at each end (18). Four human α -actinin genes have been described. The *ACTN2* and *ACTN3* genes are expressed in skeletal muscle (19), and *ACTN2* is also expressed in cardiac muscle. The *ACTN2* and *ACTN3* proteins cross-link actin filaments into tight bundles (18). The *ACTN1* gene is expressed ubiquitously (20), and its product interacts with adhesion molecules such as β 1 integrins (21, 22), ICAM-1 (23), or the cadherin/catenin complex (24). It seems to play a role in stabilizing cell adhesion and regulating cell shape and cell motility (22). The *ACTN4* gene, which is also expressed ubiquitously, was cloned recently by screening a λ gt11 cDNA expression library with a new anti- α -actinin mAb, the pattern of staining of which differed from that of an anti- α -actinin-1 antibody (16).

The precise role of α -actinin-4 is still unclear. Mutations in the gene were recently reported to cause familial focal segmental glomerulosclerosis, a common, nonspecific renal lesion characterized by increased urinary protein excretion and decreasing kidney function leading to renal failure. A lysine-to-glutamate substitution at residue 228, a threonine-to-isoleucine substitution at residue 232, and a serine-to-proline substitution at residue 235 were identified in three distinct families affected with an autosomal dominant form of the disease. *In vitro*, filamentous actin was shown to bind mutated α -actinin-4 more strongly than wild-type α -actinin-4 (25).

Cell adhesion plays a critical role in malignant transformation through dynamic interactions between the extracellular matrix and the actin cytoskeleton (26). Early studies (27) of transformed cells

showed a direct association between disorganization of the cytoskeleton and tumorigenicity or invasive capacity. It has been shown that the expression of actin-associated proteins such as α -actinin-1 was decreased in cancer cells and that increased expression after transfection could reduce or abrogate metastatic potential (28, 29). Along the same line, the human *ACTN4* gene was recently reported (30) to suppress tumorigenicity of human neuroblastoma cells, suggesting that it could be a tumor suppressor gene. A high level of *ACTN4* expression was found in nontumorigenic neuroblastoma cell variants, but little or no expression was observed in malignant neuroblasts. In addition, transfected clones of highly malignant neuroblastoma stem cells that expressed higher levels of *ACTN4* showed decreased or abrogated tumorigenicity, together with decreased anchorage-independent growth ability and decreased expression of the *N-myc* proto-oncogene (30).

The point mutation that we describe here in the *ACTN4* gene is the second mutation that is found in a gene encoding an actin-binding protein and that leads to the expression of a tumor-specific antigen recognized by autologous CTL. A mutation in the gene coding for β -catenin, which binds actin and members of the cadherin family of cell surface adhesion, was shown to encode an antigen that was specifically recognized on a melanoma by CTL derived from TIL (31). Subsequently, abnormally high amounts of β -catenin and missense mutations in the β -catenin gene resulting in stabilization of the protein were identified in several melanoma cell lines (32). Furthermore, mutations in the α -catenin (33) and β -catenin (34) genes have been found in tumor cells and appeared to be associated with a loss of cell adhesiveness. Mutations in actin-binding proteins, including α -actinins, may be involved, therefore, in the development of a malignant phenotype. We do not know whether the α -actinin-4 mutation described in this report affects the function of the protein, but a participation of this mutation in the phenotype of IGR-Heu cells cannot be excluded. However, it should be noted that similar amounts of α -actinin-4 protein were detected in IGR-Heu and Heu-EBV B-cell lines as shown by intracytoplasmic immunofluorescence analysis with the anti- α -actinin-4 mAb NCC-Lu-632, (generously provided by Dr. S. Hirohashi; Ref. 16; data not shown).

In patient Heu, the tumor-specific anti- α -actinin-4 CTLs were found in TILs, where they appeared to be clonally expanded (4), and in blood several years later. It is worth noting that, although the frequency of anti- α -actinin-4 CTLs found in blood samples was low (less than 10^{-4} among the CD8 cells), these CTLs were repeatedly detectable in the absence of clinical signs of cancer. They may

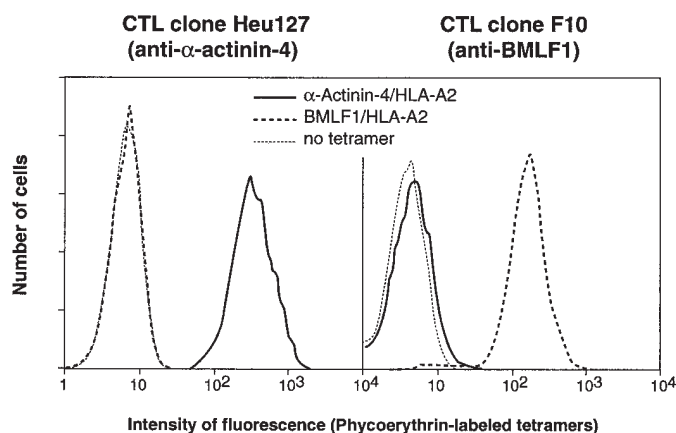


Fig. 4. Labeling anti- α -actinin-4 CTLs with tetramers. CTL clones Heu127 and F10, the latter recognizing a peptide derived from the EBV lytic protein BMLF1 and presented on HLA-A2 molecules, were incubated for 15 min at 25°C with the indicated tetramers. The cells were washed, fixed with paraformaldehyde, and analyzed by flow cytometry.

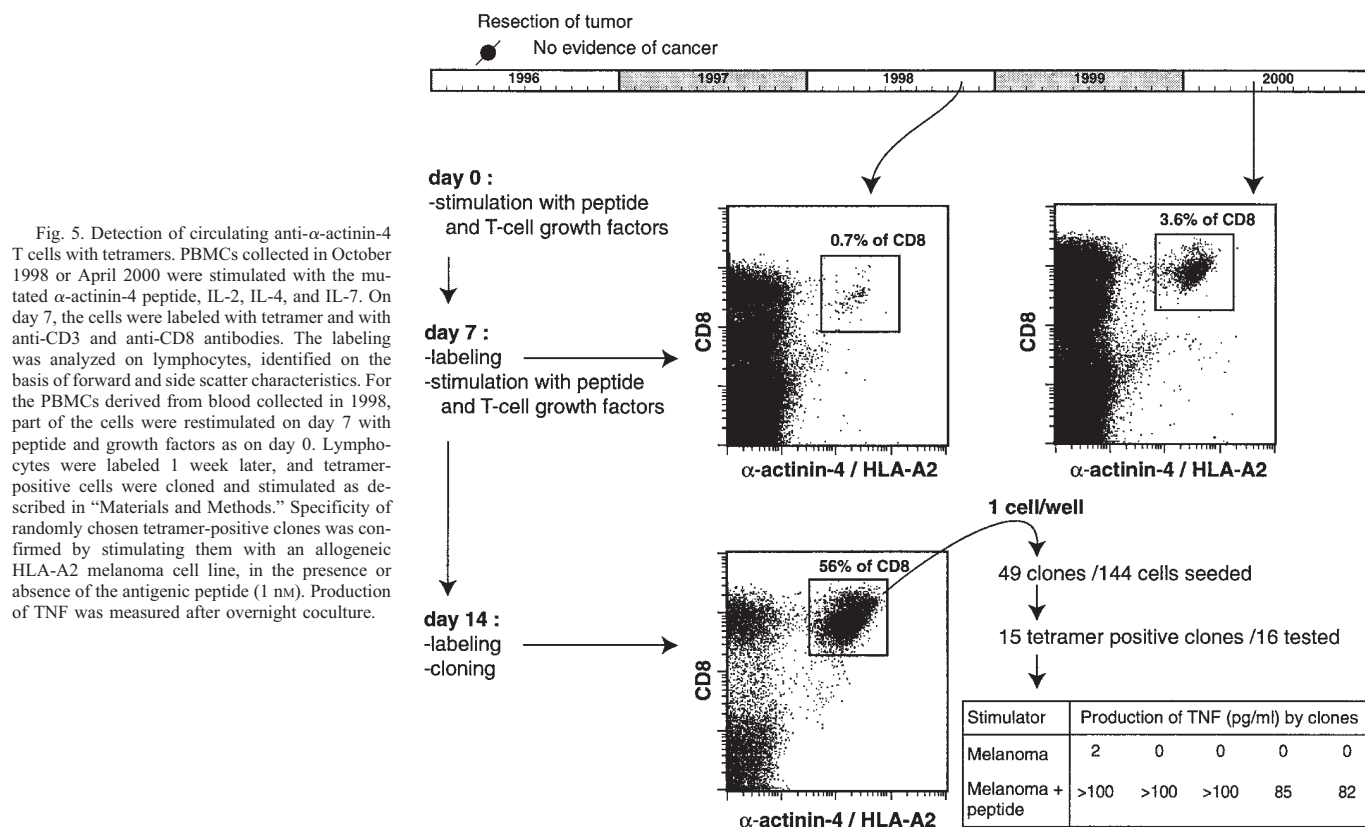


Fig. 5. Detection of circulating anti-α-actinin-4 T cells with tetramers. PBMCs collected in October 1998 or April 2000 were stimulated with the mutated α-actinin-4 peptide, IL-2, IL-4, and IL-7. On day 7, the cells were labeled with tetramer and with anti-CD3 and anti-CD8 antibodies. The labeling was analyzed on lymphocytes, identified on the basis of forward and side scatter characteristics. For the PBMCs derived from blood collected in 1998, part of the cells were restimulated on day 7 with peptide and growth factors as on day 0. Lymphocytes were labeled 1 week later, and tetramer-positive cells were cloned and stimulated as described in "Materials and Methods." Specificity of randomly chosen tetramer-positive clones was confirmed by stimulating them with an allogeneic HLA-A2 melanoma cell line, in the presence or absence of the antigenic peptide (1 nM). Production of TNF was measured after overnight coculture.

correspond to long-lived memory cells, derived from a much larger pool of CTLs that was present in the tumor and possibly also in blood. Alternatively, they may persist because they are regularly restimulated by micrometastases that remain undetected. An analysis of the phenotype of these circulating CTLs may help to clarify this issue. The presence of CTLs directed against a truly tumor-specific antigen in the blood of a disease-free lung cancer patient 4 years after resection of the tumor is compatible with the hypothesis that these CTLs participate in controlling the tumor.

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REFERENCES

- Van den Eynde, B., and van der Bruggen, P. T cell-defined tumor antigens. *Curr. Opin. Immunol.*, 9: 684–693, 1997.
- Marchand, M., van Baren, N., Weynants, P., Brichard, V., Dréno, B., Tessier, M.-H., Rankin, E., Parmiani, G., Arienti, F., Humblet, Y., Boulond, A., Vanwijck, R., Liénard, D., Beauduin, M., Dietrich, P.-Y., Russo, V., Kerger, J., Masucci, G., Jäger, E., De Greve, J., Atzpodien, J., Brasseur, F., Coulie, P. G., Van der Bruggen, P., and Boon, T. Tumor regressions observed in patients with metastatic melanoma treated with an antigenic peptide encoded by gene *MAGE-3* and presented by HLA-A1. *Int. J. Cancer*, 80: 219–230, 1999.
- Thurner, B., Haendle, I., Roder, C., Dieckmann, D., Keikavoussi, P., Jonuleit, H., Bender, A., Maczek, C., Schreiner, D., von den Driesch, P., Brocker, E. B., Steinman, R. M., Enk, A., Kampgen, E., and Schuler, G. Vaccination with MAGE-3A1 peptide-pulsed mature, monocyte-derived dendritic cells expands specific cytotoxic T cells and induces regression of some metastases in advanced stage IV melanoma. *J. Exp. Med.*, 190: 1669–1678, 1999.
- Echchakir, H., Vergnon, I., Dorothee, G., Grunenwald, D., Chouaib, S., and Mami-Chouaib, F. Evidence for *in situ* expansion of diverse antitumor specific cytotoxic T-lymphocyte clones in a human large cell carcinoma of the lung. *Int. Immunol.*, 12: 537–546, 2000.
- Baurain, J.-F., Colau, D., van Baren, N., Landry, C., Martelange, V., Vikkula, M., Boon, T., and Coulie, P. G. High frequency of autologous anti-melanoma CTLs directed against an antigen generated by a point mutation in a new helicase gene. *J. Immunol.*, 164: 6057–6066, 2000.
- Weynants, P., Thonnard, J., Marchand, M., Deloos, M., Boon, T., and Coulie, P. G. Derivation of tumor-specific cytolytic T cell clones from two lung cancer patients with long survival. *Am. J. Resp. Crit. Care Med.*, 159: 55–62, 1999.
- Slingluff, C. L., Cox, A. L., Stover, J. M., Jr., Moore, M. M., Hunt, D. F., and Engelhard, V. H. Cytotoxic T-lymphocyte response to autologous human squamous cell cancer of the lung: epitope reconstitution with peptides extracted from HLA-Aw68. *Cancer Res.*, 54: 2731–2737, 1994.
- Yoshino, I., Goedegebuure, P. S., Peoples, G. E., Parikh, A. S., DiMaio, J. M., Lysterly, H. K., Gazdar, A. F., and Eberlein, T. J. HER2/neu-derived peptides are shared antigens among human non-small cell lung cancer and ovarian cancer. *Cancer Res.*, 54: 3387–3390, 1994.
- Hogan, K. T., Eisinger, D. P., Cupp, S. B., III, Lekstrom, K. J., Deacon, D. D., Shabanowitz, J., Hunt, D. F., Engelhard, V. H., Slingluff, C. L., and Ross, M. M. The peptide recognized by HLA-A68.2-restricted, squamous cell carcinoma of the lung-specific cytotoxic T lymphocytes is derived from a mutated *elongation factor 2* gene. *Cancer Res.*, 58: 5144–5150, 1998.
- Gomi, S., Nakao, M., Niiya, F., Imamura, Y., Kawano, K., Nishizaka, S., Hayashi, A., Sobao, Y., Oizumi, K., and Itoh, K. A *cyclophilin B* gene encodes antigenic epitopes recognized by HLA-A24-restricted and tumor-specific CTLs. *J. Immunol.*, 163: 4994–5004, 1999.
- Kawano, K., Gomi, S., Tanaka, K., Tsuda, N., Kamura, T., Itoh, K., and Yamada, A. Identification of a new endoplasmic reticulum-resident protein recognized by HLA-A24-restricted tumor-infiltrating lymphocytes of lung cancer. *Cancer Res.*, 60: 3550–3558, 2000.
- Asselin-Paturel, C., Echchakir, A., Carayol, G., Gay, F., Opolon, P., Grunenwald, D., Chouaib, S., and Mami-Chouaib, F. Quantitative analysis of Th1, Th2 and TGF-β1 cytokine expression in tumor. TIL and PBL of non-small cell lung cancer patients. *Int. J. Cancer*, 77: 7–12, 1998.
- Espevik, T., and Nissen-Meyer, J. A highly sensitive cell line, WEHI 164 clone 13, for measuring cytotoxic factor/tumor necrosis factor from human monocytes. *J. Immunol. Methods*, 95: 99–105, 1986.
- Hansen, M. B., Nielsen, S. E., and Berg, K. Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. *J. Immunol. Methods*, 119: 203–210, 1989.
- Altman, J. D., Moss, P. A. H., Goulder, P. J. R., Barouch, D. H., McHeyzer-Williams, M. G., Bell, J. I., McMichael, A. J., and Davis, M. M. Phenotypic analysis of antigen-specific T lymphocytes. *Science (Wash. DC)*, 274: 94–96, 1996.
- Honda, K., Yamada, T., Endo, R., Ino, Y., Gotoh, M., Tsuda, H., Yamada, Y., Chiba, H., and Hirohashi, S. Actinin-4, a novel actin-bundling protein associated with cell motility and cancer invasion. *J. Cell Biol.*, 140: 1383–1393, 1998.
- Davidson, M. D., and Critchley, D. R. α-actinins and the DMD protein contain spectrin-like repeats. *Cell*, 52: 159–160, 1988.

18. Djinojic-Carugo, K., Young, P., Gautel, M., and Saraste, M. Structure of the α -actinin rod: molecular basis for cross-linking of actin filaments. *Cell*, *98*: 537–546, 1999.
19. Beggs, A. H., Byers, T. J., Knoll, J. H., Boyce, F. M., Bruns, G. A., and Kunkel, L. M. Cloning and characterization of two human skeletal muscle α -actinin genes located on chromosomes 1 and 11. *J. Biol. Chem.*, *267*: 9281–9288, 1992.
20. Millake, D. B., Blanchard, A. D., Patel, B., and Critchley, D. R. The cDNA sequence of a human placental α -actinin. *Nucleic Acids Res.*, *17*: 6725, 1989.
21. Otey, C. A., Pavalko, F. M., and Burridge, K. An interaction between α -actinin and the β 1 integrin subunit *in vitro*. *J. Cell Biol.*, *111*: 721–729, 1990.
22. Otey, C. A., Vasquez, G. B., Burridge, K., and Erickson, B. W. Mapping of the α -actinin binding site within the β 1 integrin cytoplasmic domain. *J. Biol. Chem.*, *268*: 21193–21197, 1993.
23. Carpen, O., Pallai, P., Staunton, D. E., and Springer, T. A. Association of intercellular adhesion molecule-1 (ICAM-1) with actin-containing cytoskeleton and α -actinin. *J. Cell Biol.*, *118*: 1223–1234, 1992.
24. Knudsen, K. A., Peralta Soler, A., Johnson, K. R., and Wheelock, M. J. Interaction of α -actinin with the cadherin/catenin cell-cell adhesion complex via α -catenin. *J. Cell Biol.*, *130*: 67–77, 1995.
25. Kaplan, J. M., Kim, S. H., North, K. N., Rennke, H., Correia, L. A., Tong, H. Q., Mthis, B. J., Rodriguez-Pérez, J. C., Allen, P. G., Beggs, A. H., and Pollak, M. R. Mutations in ACTN4, encoding α -actinin-4, cause familial focal segmental glomerulosclerosis. *Nat. Genet.*, *24*: 251–256, 2000.
26. Hynes, R. O. Specificity of cell adhesion in development: the cadherin superfamily. *Curr. Opin. Genet. Dev.*, *4*: 621–624, 1992.
27. Ben-Ze'ev, A. Cytoskeletal and adhesion proteins as tumor suppressors. *Curr. Opin. Cell Biol.*, *9*: 99–108, 1997.
28. Janmey, P. A., and Chaponnier, C. Medical aspects of the actin cytoskeleton. *Curr. Opin. Cell Biol.*, *7*: 111–117, 1995.
29. Glück, U., Kwiatkowski, D. J., and Ben-Ze'ev, A. Suppression of tumorigenicity in simian virus 40-transformed 3T3 cells transfected with α -actinin cDNA. *Proc. Natl. Acad. Sci. USA*, *90*: 383–387, 1993.
30. Nikolopoulos, S. N., Spengler, B. A., Kisselbach, K., Evans, A. E., Biedler, J. L., and Ross, R. A. The human non-muscle α -actinin protein encoded by the *ACTN4* gene suppresses tumorigenicity of human neuroblastoma cells. *Oncogene*, *19*: 380–386, 2000.
31. Robbins, P. F., El-Gamil, M., Li, Y. F., Kawakami, Y., Loftus, D., Appella, E., and Rosenberg, S. A. A mutated β -catenin gene encodes a melanoma-specific antigen recognized by tumor infiltrating lymphocytes. *J. Exp. Med.*, *183*: 1185–1192, 1996.
32. Rubinfeld, B., Robbins, P., El-Gamil, M., Albert, I., Porfiri, E., and Polakis, P. Stabilization of β -catenin by genetic defects in melanoma cell lines. *Science (Wash. DC)*, *275*: 1790–1792, 1997.
33. Oda, T., Kanai, Y., Shimoyama, Y., Nagafuchi, A., Tsukita, S., and Hirohashi, S. Cloning of the human α -catenin cDNA and its aberrant mRNA in a human cancer cell line. *Biochem. Biophys. Res. Commun.*, *193*: 897–904, 1993.
34. Kawanishi, J., Kato, J., Fujii, S., Watanabe, N., and Niitsu, Y. Loss of E-cadherin-dependent cell-cell adhesion due to mutation of the β -catenin gene in a human cancer cell line, HSC-39. *Mol. Cell. Biol.*, *15*: 1175–1181, 1995.