A Mutated HLA-A2 Molecule Recognized by Autologous Cytotoxic T Lymphocytes on a Human Renal Cell Carcinoma

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Summary

Many human tumor cells have been shown to express antigens that are recognized by autologous cytotoxic T lymphocytes (CTL) and the molecular nature of a number of melanoma antigens has been defined recently. Here we describe the characterization of an antigen recognized on a renal cell carcinoma by autologous CTL clones. This antigen is encoded by the *HLA-A2* gene present in the tumor cells. The sequence of this gene differs from the *HLA-A2* sequence found in autologous peripheral blood lymphocytes by a point mutation that results in an arginine to isoleucine exchange at residue 170, which is located on the α-helix of the α2 domain. Transfection experiments with the normal and mutated *HLA-A2* cDNA demonstrated that this amino acid replacement was responsible for the recognition of the HLA-A2 molecule expressed on the tumor cells. The mutant *HLA-A2* gene was also detected in the original tumor tissue from the patient, excluding the possibility that the mutation had appeared in vitro. Thus, HLA class I molecules carrying a tumor-specific mutation can be involved in the recognition of tumor cells by autologous CTL.

In the last five years, knowledge of human tumor antigens recognized by autologous CTL has increased considerably. So far, genetic and biochemical approaches have led to the molecular identification of three classes of antigens. The first class comprises antigens encoded by genes that are expressed in various tumors of different histological origins, but not in normal tissues other than testis, such as *MAGE-1* (1, 2), *MAGE-3* (3), *BAGE* (4), and *GAGE-1/2* (5). The second class represents differentiation antigens encoded by genes that are only expressed in melanoma and normal melanocytes, like tyrosinase (6), Melan-A^{MART-1} (7, 8), $gp100^{Pmel17}$ (9, 10), and $gp75^{TRP1}$ (11). The third class includes antigens produced by unique point mutations in genes that are ubiquitously expressed, such as *MUM-1* (12) and *CDK4* (13). In both cases, the antigenic peptide is encoded by the mutated region of the gene.

Both renal cell carcinoma (RCC)[1] and melanoma are considered potentially immunogenic in humans. Both types of tumor are often infiltrated by T lymphocytes, and metastatic lesions were found to regress spontaneously in a small but significant number of cases (14–18). Durable tumoral responses were observed after IL-2 treatment in 15–20% of cases, and some of them may result from the elicitation of T cell responses (19, 20). This suggests that kidney tumors, like melanomas, express antigens that can be recognized by autologous T lymphocytes. However, our knowledge of tumor-specific antigens expressed on RCC is scarce. In contrast to melanoma, it proved difficult to adapt RCC to in vitro culture conditions, and to generate stable CTL clones for the identification of antigens present on this type of tumor. In addition, genes *MAGE-1*, *MAGE-3*, *BAGE*, and *GAGE-1/2* are expressed in various tumors but not in RCC.

Here we report the identification of an antigen that is recognized by autologous CTL on human RCC cell line LB996-RCC. The antigen is encoded by a mutated *HLA-A2* gene.

Materials and Methods

Cell Lines. RCC cell line LB996-RCC was derived from the primary tumor of 67-yr-old Caucasian patient LB996 (HLA-A2, -A11, -B18, -B44, -Cw1, and -Cw*1601) and grown as previously described (21). This patient died of pulmonary embolism 7 wk after nephrectomy for a stage III RCC that invaded the inferior vena cava. Lymphoblastoid cell line LB996-EBV was derived from the PBL of patient LB996 by standard techniques. CTL clone 314/4 was derived from LB996-PBL which were stimulated in vitro with irradiated autologous LB996-RCC cells. This CTL clone expressed both CD4 and CD8 molecules. Other CTL clones

[1] *Abbreviations used in this paper:* RCC, renal cell carcinoma; RT, reverse transcription.

obtained similarly expressed only CD8 and were also directed against HLA-A*0201-R170I (data not shown). RFLP analysis confirmed that LB996-RCC and LB996-PBL originated from the same patient (data not shown). HeLa-S3 cells were obtained from the American Type Culture Collection (Rockville, MD). They were transfected by the calcium phosphate precipitate method with *HLA-A*0201-R170I* or *HLA-A*0201* cDNA cloned individually into plasmid pcDNA3 (Invitrogen Corp., San Diego, CA) which contains the neomycin resistance gene. Clonal sublines were isolated from transfected populations resistant to G418 (2 mg/ml).

Cytotoxicity Assay. The lytic activity of CTL was tested in a chromium release assay as previously described (22). Briefly, 1,000 chromium-labeled cells in 100 μl were incubated in 96-well microplates with an equal volume of CTL at different effector to target ratios. Chromium release was measured after 4 h of incubation.

CTL Stimulation Assay. 2,500 CTL were added to microwells containing stimulator cells in 100 μl of Iscove medium (GIBCO BRL, Gaithersburg, MD) supplemented with 10% human serum and 25 U/ml r-hu-IL-2. After 18 h, the supernatant was collected and its TNF content determined by testing the cytotoxic effect on WEHI-164 clone 13 cells (23) in a MTT colorimetric assay (24). Inhibition with mAbs W6/32 (anti-HLA class I; 25), L243 (anti-HLA-DR; 26), BB7.2 (anti-HLA-A2; 27), B.9.4.1 (anti-CD8), and 13B8.2 (anti-CD4) (donated by Dr. D. Olive, INSERM U119, Marseille, France) was performed by addition of a 1/20 dilution of ascites to the test.

Construction of the cDNA Library. Total RNA was extracted by the guanidine-isothiocyanate procedure (28) from LB996-RCC cells that had undergone 25 in vitro passages after surgical removal from patient LB996. Poly(-A)$^+$ RNA enriched by an oligo(dT)-cellulose column was converted to cDNA with the Superscript Choice System (GIBCO BRL) using an oligo(dT) primer containing a NotI site at its 5′ end. The cDNA was ligated to BstXI adaptors, digested with NotI, and inserted into the BstXI and NotI sites of expression vector pcDNA3. Recombinant plasmids were electroporated into *Escherichia coli* Top10F′ and selected with ampicillin (100 μg/ml). The library was divided into 400 pools of 100 cDNA clones. Each pool of bacteria was amplified to saturation and plasmid DNA was extracted using the QIAprep 8 plasmid kit (Qiagen GmbH, Hilden, Germany).

Transfection of COS-7 Cells for Screening the cDNA Library. Transient transfection was performed by the DEAE-dextran-chloroquine method (29). Briefly, 1.5 × 10^4 COS-7 cells were transfected with 100 ng of plasmid DNA from a pool from the cDNA library, and 100 ng of plasmid pcDNA3 containing the *HLA-A*0201* gene that was derived from an unrelated individual (30). Transfected COS-7 cells were tested in a CTL stimulation assay after 48 h.

Cloning of the HLA-A2 cDNA from LB996-PBL. Total RNA was extracted from LB996-PBL by the guanidine-isothiocyanate procedure (28). Reverse transcription (RT) was performed on 2 μg of total RNA in a 20-μl reaction volume containing 4 μl of 5× reverse transcriptase buffer (GIBCO BRL), 1 μl each of 10 mM dNTP, 2 μl of a 20-μM solution of oligo$(dT)_{15}$ primer, 20 U of RNasin (Promega Corp., Madison, WI), 2 μl of 0.1 M dithiothreitol, and 200 U of Moloney murine leukemia virus reverse transcriptase (GIBCO BRL). The reaction was incubated at 42°C for 60 min. One-twentieth of the cDNA product was then supplemented with 10 μl of 10× polymerase buffer, 2 μl each of 10 mM dNTP, 5 μl each of 20 μM primer solutions, 2.5 U of PFU DNA polymerase (Stratagene Inc., La Jolla, CA) and water to a final volume of 100 μl. PCR amplification was carried out using forward primer 5′-GGCGAATTCGGACTCAGAATCTCCCC-AGACGCCGAG and reverse primer 5′-CCCGAATTCTCT-CAGTCCCTCACAAGGCAGCTGTC that hybridize to the 5′ and 3′ untranslated regions of all HLA class I genes respectively, and that both contain an EcoRI site (31). An initial denaturation step was performed for 4 min at 94°C, followed by 30 cycles (1 min at 94°C, 5 s at 62°C, and 3 min at 75°C) and 5 additional cycles (1 min at 94°C, 5 s at 62°C, and 5 min at 75°C). The amplification finished with 10 min at 75°C. The PCR product was digested with EcoRI and inserted into the EcoRI site of pcDNA3.

DNA Sequence Analysis. DNA sequencing was performed by the dideoxy-chain termination method (T7 Sequencing Kit; Pharmacia, Uppsala, Sweden) using specific oligonucleotides as primers. The computer search for sequence homology was performed with the blast@ncbi.nlm.nih.gov. program, (National Library of Medicine, NIH, Bethesda, MD).

*PCR Amplification of HLA-A*0201-R170I and HLA-A*0201 Gene Sequences.* Total RNA extraction, conversion to cDNA, and PCR amplifications were performed as described in a previous section with minor modifications; 1 U of DynaZyme™ (Finnzymes OY, Espoo, Finland) instead of PFU DNA polymerase was used. Amplification of the *HLA-A2*0201-R170I* gene was carried out over 31 cycles (30 s at 94°C, 30 s at 63°C, and 1 min at 72°C), and that of the *HLA-A*0201* gene was performed for 32 cycles (30 s at 94°C, 30 s at 64°C, and 1 min at 72°C) using either reverse primer 5′-CCTTCCCGTTCTCCAGGTATA, corresponding to nucleotides 581–601 of *HLA-A*0201-R170I*, or 5′-CTTCCCGTTCTCCAGGTATC, corresponding to nucleotides 581–600 of *HLA-A*0201*, respectively, and forward primer 5′-GGACGGGGAGACACGGAAA, corresponding to nucleotides 252–270 of both genes. The underlined nucleotides of the reverse primers determine the sequence specificity.

Cytofluorometric Analysis. 5 × 10^5 cells were stained in 100 μl of PBS containing 3% FCS and 20 mM NaN_3 for 10 min at 4°C for each step. The following antibodies were used: FITC-conjugated goat anti-mouse Ig (Becton Dickinson & Co., Mountain View, CA), HLA-A2–specific mAbs CR11-351 (32) and MA2.1 (33), BB7.2 (27) and PA2.1 (34). Cells were analyzed on a FACScan® (Becton Dickinson & Co.) using four logarithmic scales. Data were only collected from viable cells gated by a combination of forward light scatter and 90° side scatter.

Results

CTL Clones Specific for RCC Cell Line LB996-RCC. By stimulating PBL from patient LB996 with irradiated cells from autologous RCC line LB996-RCC, we isolated a panel of CTL clones that specifically lysed the tumor cells, but not an autologous EBV-transformed B cell line or NK cell target K562. Results obtained with representative CTL clone 314/4 are shown (Fig. 1 *A*).

CTL clone 314/4 produced TNF when stimulated with LB996-RCC cells, and this TNF production was blocked by a mAb that binds to all HLA class I molecules (Fig. 1 *B*). Partial inhibition of TNF production was also observed in the presence of mAbs directed against HLA-A2 or against CD8, suggesting that the target antigen is presented by HLA-A2.

Identification of a cDNA Coding for the Antigen Recognized by CTL 314/4. To clone the gene conferring recognition by CTL 314/4, we prepared a cDNA library from

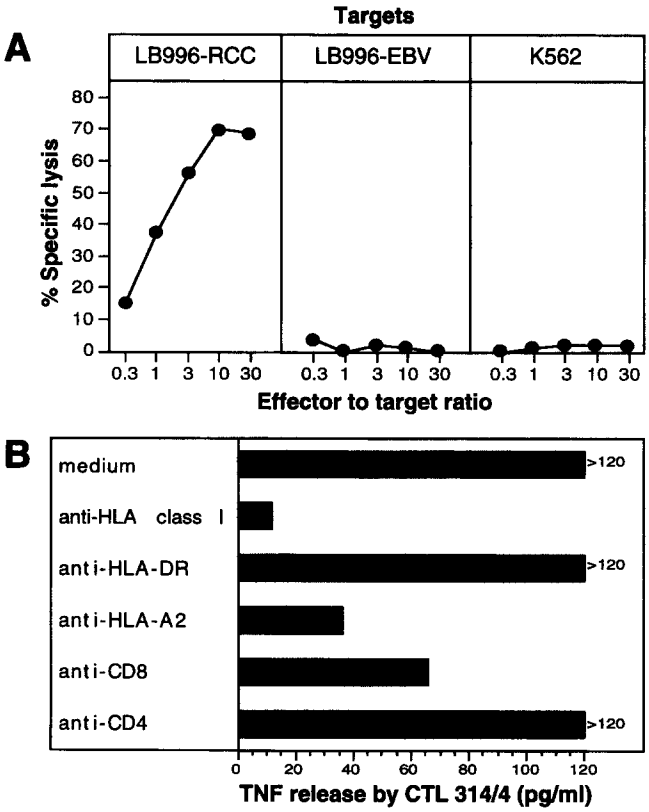

Figure 1. Specificity of CTL 314/4. (*A*) Specific lysis of autologous LB996-RCC cells by CTL 314/4. LB996-RCC cells, autologous EBV-transformed lymphoblastoid line LB996-EBV, and NK cell–sensitive line K562 were used as target cells. Chromium release was measured after 4 h. (*B*) Recognition of LB996-RCC cells by CTL 314/4 is inhibited by anti-HLA class I, anti-HLA-A2 and anti-CD8 mAbs. LB996-RCC cells were used to stimulate CTL 314/4 in the presence of mAbs with the specificities indicated. After 18 h of coculture, production of TNF by CTL 314/4 was measured by testing toxicity of the supernatants for TNF-sensitive WEHI-164.13 cells.

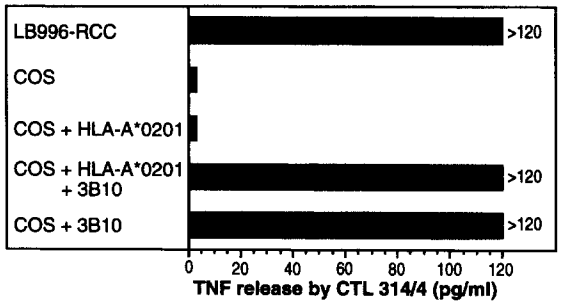

Figure 2. Stimulation of CTL 314/4 by COS-7 cells transfected with cDNA clone 3B10. COS-7 cells were transiently transfected with the indicated cDNA cloned individually into pcDNA3. *HLA-A*0201* cDNA was previously cloned from an unrelated individual and cDNA 3B10 was cloned from LB996-RCC cells. After 18 h of coculture with transfected cells, production of TNF by CTL 314/4 was measured by testing toxicity of the supernatants for TNF-sensitive WEHI-164.13 cells. Control stimulator cells included autologous LB996-RCC and untransfected COS-7 cells.

RNA of LB996-RCC cells in expression vector pcDNA3. We divided the library into 400 pools of 100 bacteria and transfected duplicate microcultures of COS-7 cells with DNA from each pool together with a plasmid coding for HLA-A*0201. We tested the transfected cells 48 h later for their ability to induce TNF release by CTL 314/4. Three cDNA pools proved positive. By subcloning one of them, we isolated three cDNA clones that transferred the expression of the antigen recognized by CTL 314/4. The result of representative cDNA clone 3B10 is shown (Fig. 2). Surprisingly, the expression of HLA-A*0201 was not necessary as COS-7 cells transfected with clone 3B10 alone were able to stimulate TNF production.

A Mutated HLA-A2 Molecule is Recognized by RCC-specific CTL Clone 314/4. The finding that the product of cDNA 3B10 alone was recognized by CTL 314/4 (Fig. 2), and the capacity of anti-HLA-A2 mAbs to block the recognition of LB996-RCC cells by CTL 314/4 (Fig. 1 *B*) indicated that cDNA 3B10 itself might code for a HLA-A2 molecule. In fact, the sequence of cDNA 3B10 proved identical to that of *HLA-A*0201* with the exception of one nucleotide: the guanine (G) at position 581 of *HLA-A*0201* was replaced by a thymine (T), which resulted in an arginine (R) to isoleucine (I) exchange at residue 170 (Fig. 3 *A*). Accordingly, the amino acid replacement was referred to as R170I, and the *HLA-A2* gene expressed by LB996-RCC cells was named *HLA-A*0201-R170I.* As shown in Fig. 4, residue 170 is located on the α-helix of the α2 domain.

This new HLA-A2 sequence did not correspond to a previously described HLA-A2 allele. Therefore, to determine whether HLA-A*0201-R170I represented a new HLA-A2 allele, or resulted from a somatic point mutation in tumor LB996-RCC, we analyzed the corresponding sequence in normal cells from the same patient. We amplified by RT-PCR the coding region of the *HLA-A2* gene expressed by PBL LB996-PBL, and cloned it into expression vector pcDNA3. This *HLA-A2* sequence proved identical to that of *HLA-A*0201*, thus differing from the *HLA-A*0201-R170I* sequence by one nucleotide (Fig. 3, *A* and *B*). Since patient LB996 was HLA-typed A2 and A11, the two HLA-A2 sequences could not represent two alleles of the HLA-A2 locus. We concluded that the R170I replacement was created by a somatic mutation in the tumor cells.

To confirm the role of this mutation in creating the antigen recognized by CTL 314/4, we transfected COS-7 cells with plasmids containing the *HLA-A2* sequence isolated either from LB996-PBL or from tumor cells LB996-RCC. As shown in Fig. 5 *A*, CTL 314/4 only produced TNF when stimulated with COS-7 cells that expressed HLA-A*0201-R170I. The possibility that the high replication rate of the transfected *HLA-A2* genes in COS-7 cells caused nonspecific stimulation of CTL 314/4 was further excluded by the finding that HeLa cells stably transfected with *HLA-A*0201-R170I* cDNA were lysed by CTL 314/4, whereas stable HeLa transfectants expressing the *HLA-A*0201* cDNA were not (Fig. 5 *B*).

Autologous EBV-transformed B cells pulsed with different peptides derived from the mutant region of HLA-A*0201-R170I were not recognized by CTL 314/4, suggesting that

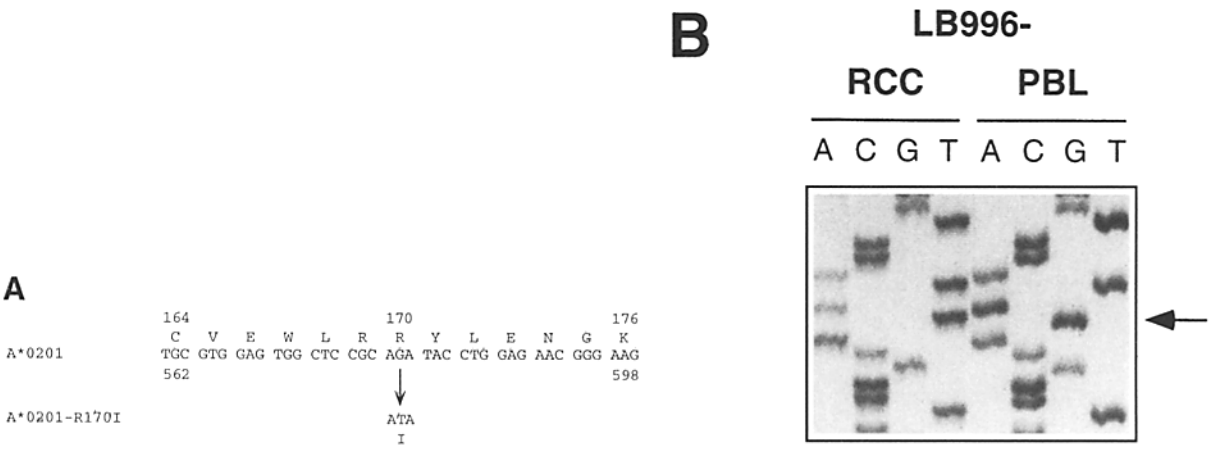

Figure 3. A point mutation leads to an amino acid exchange in the coding region of the *HLA-A2* gene expressed by LB996-RCC cells. (*A*) Alignment of the partial nucleotide sequence and the deduced protein sequence of HLA-A*0201 (*top*) in relation to the mutation occurring in LB996-RCC cells (*bottom*). The sequence of the *HLA-A2* cDNA cloned from LB996-PBL is identical to that of *HLA-A*0201*. The G to T transition at position 581 of *HLA-A*0201* leads to an arginine to isoleucine exchange at residue 170; correspondingly, the mutated gene is named *HLA-A*0201-R170I*. (*B*) Representative autoradiography showing the sequence ladder around nucleotide 581 of the *HLA-A2* cDNA from LB996-RCC cells and LB996-PBL. Plasmids pcDNA3 containing the *HLA-A2* cDNA cloned from either RCC (*left*) or PBL (*right*) were sequenced by the dideoxy-chain termination method using oligonucleotide 5′-AAGGATTACATCGCC (nucleotides 433 to 447 of gene *HLA-A2*) as primer. The G (*PBL*) to T (*RCC*) transition at position 581 is indicated by the arrow.

the CTL recognizes the altered HLA-A2 molecule itself rather than a mutant peptide presented by autologous MHC molecules (data not shown).

The R170I Mutation Occurred In Vivo. To determine whether the R170I mutation was already present in the original tumor tissue or whether it was generated in vitro in LB996-RCC cells, we designed specific primers to selectively amplify by PCR either the normal *HLA-A*0201* or the mutated sequence. Whereas LB996-PBL and the cultured LB996-RCC cell line only contained the wild-type or the mutated sequence respectively, both sequences were found in the initial tumor tissue (Fig. 6). The weak signal for the wild-type gene is probably due to some normal cells that were surgically removed together with the tumor mass. The strong band for the mutated sequence clearly shows that the mutation occurred in vivo.

To examine whether the R170I mutation occurs frequently, 34 HLA-A2$^+$ tumor samples of different histolog-

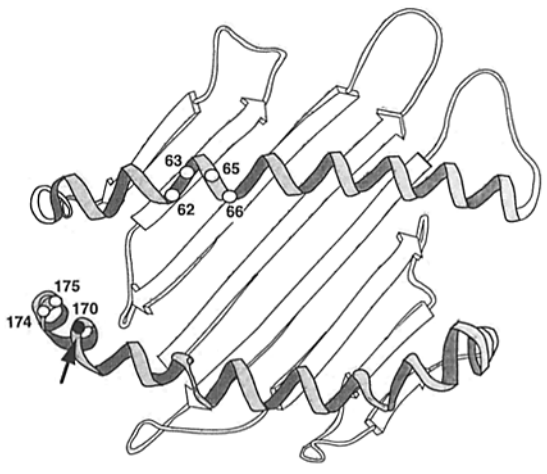

Figure 4. Schematic representation of the structure of HLA-A2. The position of residue 170 (*closed circle*) which is altered in LB996-RCC is indicated (*arrow*). (*open circles*) The positions previously known to be important for binding of mAb MA2.1.

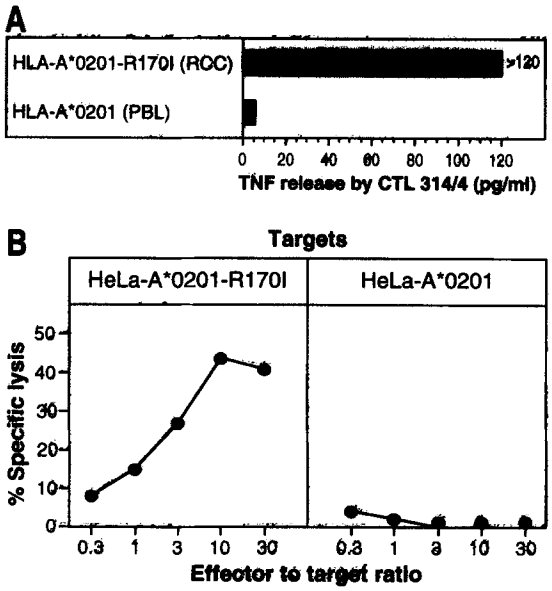

Figure 5. HLA-A*0201-R170I is recognized by CTL 314/4. (*A*) Stimulation of CTL 314/4 by COS-7 cells transfected with plasmid pcDNA3 containing the *HLA-A*0201-R170I* cDNA. The *HLA-A*0201-R170I* cDNA was cloned from a cDNA library of LB996-RCC cells, whereas the *HLA-A*0201* cDNA was cloned from LB996-PBL by RT-PCR using HLA class I–specific oligonucleotides as described in Materials and Methods. Production of TNF by CTL 314/4 was measured as described in Fig. 2. (*B*) Specific lysis by CTL 314/4 of HeLa cells expressing the *HLA-A*0201-R170I* cDNA. HeLa cells stably transfected with either the *HLA-A*0201-R170I* or the *HLA-A*0201* cDNA were used as target cells. Chromium release was measured after 4 h. The HeLa transfectants expressed the same density of HLA-A2 molecules at the cell surface as analyzed by flow cytometry with mAb CR11-351 (data not shown).

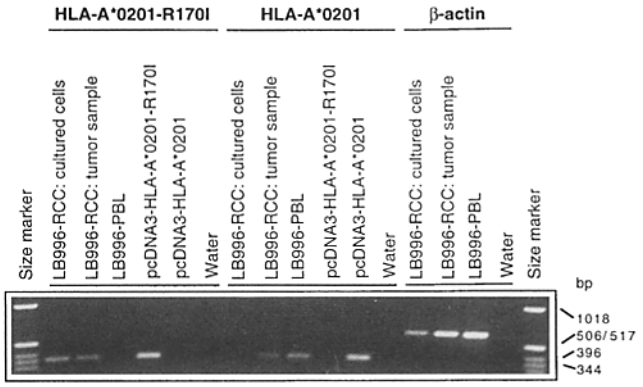

Figure 6. Detection of the point mutation by RT-PCR in the original tumor sample of patient LB996. Total RNA isolated from cultured LB996-RCC cells, from the original LB996-RCC tumor sample, and from LB996-PBL was subjected to RT. The resulting cDNA products were used as templates for selective PCR amplification of *HLA-A*0201-R170I* (lanes *1–6*) or *HLA-A*0201* (lanes *7–12*). RNA integrity was checked by RT and amplification of β-actin mRNA (lanes *13–16*). Control templates included plasmid pcDNA3 (25 pg) containing cDNA *HLA-A*0201-R170I* or *HLA-A*0201*.

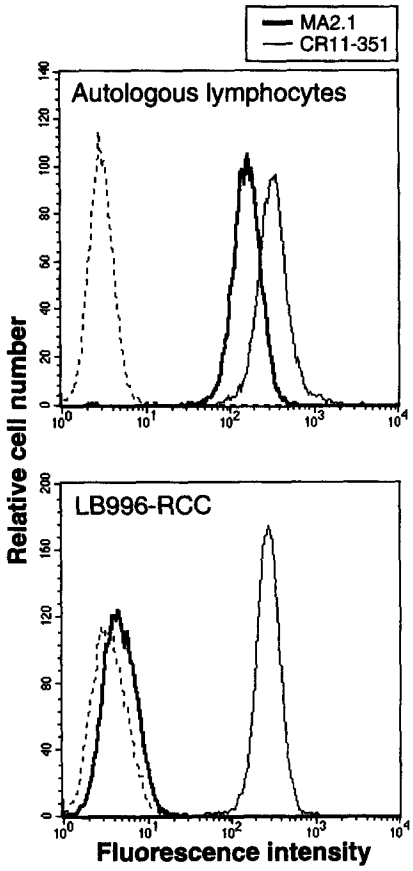

Figure 7. Effect of the arginine to isoleucine exchange at residue 170 of HLA-A*0201 on the binding of HLA-A2–specific mAbs. Autologous lymphocytes represented by CTL 314/4 (*top*) and LB996-RCC cells (*bottom*) were incubated with mAbs CR11-351 (*thin line*) or MA2.1 (*thick line*), respectively. After washing, the cells were incubated with FITC-conjugated goat anti–mouse Ig antibodies, washed again, and analyzed on a FACScan® using four logarithmic scales. (*dashed lines*) Negative control stainings using the fluorescent conjugate alone are shown. Binding of mAbs BB7.2 and PA2.1 was identical to that of CR11-351 (data not shown).

ical origins were screened by RT-PCR. All samples proved negative for the mutated sequence (data not shown).

*Recognition of the Mutated HLA-A*0201 Molecule by mAbs.* To examine the influence of the R170I mutation on the structure of the HLA-A*0201 molecule, a panel of HLA-A2–specific mAbs was used to detect altered antibody binding sites by flow cytometry. Whereas mAbs CR11-351, BB7.2, and PA2.1 stained lymphocytes of patient LB996 with the same intensity as the RCC line, the binding of mAb MA2.1 was strongly reduced on LB996-RCC cells when compared with autologous lymphocytes (Fig. 7). Although the binding site of mAb MA2.1 has previously been mapped to residues 62-66 on the α-helix of the α1 domain (35), the finding that residue 170, which is located on the α-helix of the α2 domain, also affects binding of MA2.1, is in agreement with a previous report (36), and suggests that the epitope is not limited to the α1 helix (Fig. 4).

Discussion

The ability of point mutations to generate new antigens recognized by T cells was first demonstrated by the analysis of tum$^-$ antigens, which appear on mouse tumor cells after in vitro mutagenesis (37). These mutations occur in genes expressed ubiquitously, and the resulting amino acid substitutions generate new antigenic peptides, either by creating an agretope enabling the mutated peptide to bind to MHC molecules, or by creating a new epitope in a peptide already able to bind (38). This mechanism was later found to be responsible for the presence of T cell antigens on both murine and human tumors, in the absence of any in vitro mutagenesis (12, 13, 39, 40). So far, all tumor antigens generated by point mutations correspond to mutated peptides presented by MHC molecules.

The antigen described here also results from a tumor-specific point mutation. However, this mutation does not create a new antigenic peptide, but rather modifies the structure of the HLA-A2 molecule which now appears to be recognized per se. The ability of altered MHC molecules to act as tumor-specific antigens has been extensively debated, starting with observations by Invernizzi and Parmiani (41, 42). The mutated HLA-A2 molecule described here is the first instance of a somatic point mutation changing an amino acid in a MHC class I molecule, thereby transforming it into a target structure that is recognized by autologous CTL. Because this mutation is present in tumor cells and not in autologous normal cells, the antigen is strictly tumor specific and therefore represents an ideal target for cancer immunotherapy. On the other hand, since the mutation, and consequently the antigen, are unique to an individual tumor, no vaccine of general use can be based on it.

The structural basis for recognition of the altered HLA-

A2 molecule by the TCR is not clear: either the mutant HLA-A2 binds a different set of endogenous peptides, one of which is recognized by the receptor, or the receptor interacts directly with the altered HLA-A2 molecule independently of a specific peptide. Preliminary experiments suggest that acid elution of peptides bound to HLA molecules of LB996-RCC cells does not affect recognition by CTL 314/4, whereas recognition by a control CTL was reduced (data not shown). This observation would tend to favor the last hypothesis.

This antigen appears to be strongly immunogenic in vitro, since all 25 LB996-RCC–specific CTL clones that were isolated recognized HLA-A*0201-R170I (data not shown). Since patient LB996 did not reject his tumor, one might ask why he failed to mount an effective antitumor immune response in vivo. There are several possible explanations of the paradox of in vitro measurable CTL reactivity without tumor response in vivo. First, tumor-specific T cells might be present in vivo but unresponsive. This might be due to the absence on tumor cells of costimulatory molecules required for efficient stimulation of T cells (for a review see reference 43). T cells infiltrating kidney tumors were shown to express reduced levels of the TCR ζ chain, which might partly explain the unresponsiveness resulting from incomplete stimulation with tumor antigens (44, 45). Such T cells could, however, respond in vitro in the presence of lymphokines replacing the missing costimulatory signal. The finding that costimulation of $CD8^+$ T cells by B7-transfected melanoma cells resulted in tumor rejection in two mouse models supports the importance of providing two signals to T lymphocytes (46, 47). Another reason for the absence of an effective antitumor response in vivo could be the selective deletion of tumor-specific high-avidity T cells due to the presence of the tumor. Similar to observations in spleen-chimeric mice (48), and in H-2 transgenic mice (49), it is possible that the remaining low-avidity T cells lack potent functional activity in vivo, but can respond in vitro under appropriate stimulatory conditions, leading to a state of split tolerance.

Regardless of the mechanism underlying the unresponsiveness in vivo, it may be possible to immunize patients against mutated antigens by using presentation by dendritic cells, or by injecting the antigen together with adjuvants or lymphokines. Although unique antigens are restricted to individual tumors, their exquisite tumor specificity would make them very safe targets for such active immunizations. In addition, transplantation studies with chemically induced mouse tumors have shown that unique antigens can be very strong immunogenic rejection antigens (38, 50). The observation that tumor cells are frequently deficient in DNA-repairing enzymes (51–55), and therefore accumulate mutations possibly generating new antigenic peptides, suggests that such unique antigens may be frequently present on human tumors. It is not impossible that future technical improvements will allow the design of individually specific vaccines based on defined unique antigens.

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