Conserved TCR usage by HLA-Cw*1601-restricted T cell clones recognizing melanoma antigens

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Abstract

In this study we determined TCR α and β chain nucleotide sequences of HLA-Cw*1601-restricted cytotoxic T lymphocyte (CTL) clones obtained from the peripheral blood lymphocytes (PBL) of a melanoma patient. These clones were previously shown to be involved in the recognition of melanoma-associated antigenic epitopes SAYGEPRKL and AARAVFLAL encoded by gene MAGE-1 and BAGE respectively. All (3/3) anti-MAGE-1 CTL clones displayed TCRBV5 usage and one clonotype was found twice, >1 year apart, in patient’s PBL. Two out of three anti-BAGE CTL clones showed the same TCRAV/AJ and TCRBV/BJ combinations and differed in the α chain CDR3 for two residues and in the β chain CDR3 for a single nucleotide which, however, did not change translation. These results suggest a pattern of TCR conservation in CTL selected for recognition of MAGE-1 or BAGE peptides on the autologous melanoma.

Recently, sequences of antigenic epitopes presented by appropriate MHC class I molecules and recognized on human melanoma by cytotoxic T lymphocytes (CTL) have been described (1). One class of melanoma-associated peptides is encoded by genes that are expressed in tumors but not in normal adult tissues except testis, and include MAGE, BAGE and GAGE genes (1). A second class is encoded by genes expressed only in melanocytes and melanomas such as Melan-A/MART-1, tyrosinase, gp100, gp75 and p15 (1). Finally, point mutations also generate antigens that are unique for an individual tumor (1). Recognition of immunogenic peptides in the context of MHC molecules is mediated by the TCR, a clonotypic heterodimeric glycoprotein localized on the surface of T lymphocytes (2). Each chain consists of a variable region, which confers the specificity of the receptor, and a constant region. TCR diversity arises in the thymus by rearrangement of variable (V), diversity (D, only for β chain) and joining (J) gene segments. The presence of multiple V, D, J germline sequences, their imprecise joining, the random addition/removal of nucleotides and the combination of two chains allow the great potential diversity necessary for the recognition of a wide range of peptide–MHC combinations (2). Nevertheless, limited heterogeneity in TCR usage by human MHC class I-restricted CTL has been reported in the recognition of specific peptides, mainly of viral origin (3–6).

As far as class I-restricted recognition of molecularly defined tumor antigens is concerned, available data are limited. A conserved TCR usage was found, both at a single patient level and in different patients, in T cell clones specific for the immunodominant epitope of the melanocyte differentiation antigen Melan-A/MART-1 restricted by the HLA-A2 molecule although different TCR recognizing the same epitope also co-exist within single individuals (7–9). Completely different TCR were instead used by three CTL clones derived from a single patient and recognizing the tumor-specific epitope MAGE-1 in association with HLA-A1 (10).

Since CTL generated against some melanoma antigens, when adoptively transferred, can mediate tumor regression in patients with metastatic disease (11,12), the identification of antigenic tumor peptides whose HLA class I-restricted recognition involves conserved TCR usage, coupled with increasing knowledge of TCR composition, may allow targeting of tumor-specific TCR for immunotherapeutic or diagnostic purposes.

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Table 1. Peptide specificity and TCRV gene usage of HLA-Cw*1601-restricted CTL clones

<table>
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<th>CTL clones</th>
<th>Datea</th>
<th>Dateb</th>
<th>Antigen</th>
<th>Peptide</th>
<th>TCRAVb</th>
<th>TCRBVb</th>
<th>Ref</th>
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<tr>
<td>26/331</td>
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<td>ND</td>
<td>13</td>
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<td>7/7/1984</td>
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<td>5</td>
<td>13</td>
</tr>
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<td>5</td>
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<td>SAYGEPRKL</td>
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<td>ND</td>
<td>13</td>
</tr>
<tr>
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<td></td>
<td>MAGE-1</td>
<td>SAYGEPRKL</td>
<td>ND</td>
<td>ND</td>
<td>13</td>
</tr>
<tr>
<td>25/244</td>
<td>3/23/1983</td>
<td></td>
<td>BAGE</td>
<td>AARAVFLAL</td>
<td>8</td>
<td>8</td>
<td>14</td>
</tr>
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<td>AARAVFLAL</td>
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<td>13</td>
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<td>3, 4</td>
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<td>14</td>
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<td>82/66</td>
<td>7/7/1984</td>
<td></td>
<td>BAGE</td>
<td>AARAVFLAL</td>
<td>ND</td>
<td>ND</td>
<td>14</td>
</tr>
</tbody>
</table>

ND, not determined in previous studies.

aDate of blood sample.
bTCRAV and BV expression was detected by PCR analysis.

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Fig. 1. TCR α and β nucleotide sequences of independent MAGE-1/HLA-Cw*1601- and BAGE/HLA-Cw*1601-restricted CTL clones. Only a representative sequence is reported when CTL clones derive from the same mixed lymphocyte-tumor cell culture and share identical nucleotide sequences (as for anti-MAGE-1/Cw*1601 clones 81/9, 81/12 and 81/20, and for anti-BAGE/Cw*1601 clones 82/82 and 82/66). The standardized TCR nomenclature proposed by the International Union of Immunological Societies has been used throughout this paper (20). TCRα and TCRγ gene subfamilies were classified according to Wilson et al. (21) and to Wei et al. (22), respectively. Sequencing of TCRBV5 and TCRBV8 amplified fragments did not allow identification of subfamily members. TCRJ segments were assigned according to Koop et al. (19), and TCRBD, BJ and BC elements according to Toyonaga et al. (23). The TCRα and TCRβ CDR3 length are defined according to Moss and Bell (24). TCR joining segment residues contributing to CDR3 are underlined.

We have previously described several T cell clones recognizing on the autologous melanoma either the MAGE-1-encoded epitope SAYGEPRKL or the BAGE-encoded epitope AARAVFLAL bound to HLA-Cw*1601 (13, 14) and derived from a single melanoma patient (MZ2). The features of these clones are summarized in Table 1. Anti-MAGE-1/Cw*1601 26/331 and anti-BAGE/Cw*1601 25/244 clones were derived from peripheral blood lymphocytes (PBL) obtained in 1983 when patient MZ2 underwent surgery for malignant melanoma metastases; the remaining CTL clones were generated from PBL obtained in 1984-86 after the patient received multiple injections of an autologous melanoma vaccine. This vaccine consisted of a mixture of cloned autologous tumor cells adapted in culture from an abdominal metastasis and surviving N-methyl-N-nitro-nitrosoguanidine mutagenic treatment (15). Expression of TCR variable gene expression, determined by PCR amplification for some of the clones, indicated a shared usage of TCRBV5 and TCRBV13 for two anti-MAGE-1/Cw*1601 CTL clones and two anti-BAGE/Cw*1601 CTL clones respectively (13, 14) (Table 1). It is of note that all but one of the tested clones displayed two Va chain transcripts.

In the present study we sought to determine nucleotide sequences encoding TCR α and β chains from all available CTL clones in order to compare junctional TCRα-J and TCRβ–BD-BJ regions encoding complementarity determining region (CDR) 3 and to identify the functional α chains.
Methods for RNA, cDNA preparation, PCR amplification and sequencing have been previously described (9,16). Briefly, total RNA was prepared from CTL clones by using RNAzol B (Cinna/Biotecx, Friendswood, TX) and first strand cDNA was synthesized with oligo(dT) and reverse transcriptase (Superscript, Gibco/BRL, Gaithersburg, MD). PCR was carried out by amplification with primers complementary to TCR V and C region sequences (16) in a 25 µl reaction mixture containing 0.5 µl of cDNA, all four dNTPs (each at 200 µM), 1 µl of each primer and 0.625 U Taq polymerase (Ampli Taq, Perkin-Elmer/Cetus, Emeryville, CA) on a DNA thermal cycler (model 9600 Gene Amp PCR system; Perkin-Elmer/Cetus). Amplification was performed for 30 cycles, each consisting of 1 min at 95°C, 30 s at 60°C and 1 min at 72°C. PCR products were then cloned into the pCR-Script SK(+) vector (pCR-Script SK(+) Cloning Kit; Stratagene, La Jolla, CA) and sequenced with Sequenase 2.0 (US Biochemicals, Cleveland, OH).

Nucleotide sequences of functional TCR α and β chains expressed by independent anti-MAGE-1/Cw*1601 CTL clones are shown in Fig. 1. CTL clones 26/331 and 82/35, obtained from PBL taken before and after tumor cell vaccination respectively (Table 1), have an identical TCR clonotype. The second a chain transcript contained a stop codon at the TCRAV10/TCRAJ45 gene segments junction and, therefore, such transcript is not functional (data not shown). The finding of shared TCRBV5 usage, including a conserved leucine at position 96 of TCRD3 CDR3 and TCRB CDR3, suggests a selection for certain TCR elements in the MAGE-1/HLA-Cw*1601-restricted T cell response of this patient.

In conclusion, two clonotypes, both using TCRBV5, were identified among three independent CTL clones recognizing the MAGE-1/HLA-Cw*1601 complex, and an almost identical TCR composition was found in two out of three BAGE/HLA-Cw*1601-directed CTL clones, implying a high degree of conservation. Despite the fact that they were obtained in a single patient and need to be confirmed in additional melanoma patients, such findings corroborate previous studies indicating a restricted use of the TCR among CTL clones recognizing the HLA-A2-restricted epitope of Melan-A/MART-1 (7,9). In addition, these results open the possibility to isolate...
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Abbreviations
- CDR: complementarity determining region
- CTL: cytotoxic T lymphocytes
- PBL: peripheral blood lymphocytes

Note
The nucleotide sequence data reported in this paper are available from EMBL GenBank under accession nos X94088–X94097.

References