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

Cinthia Farina, Pierre van der Bruggen1, Pascale Boël1, Giorgio Parmiani and Marialuisa Sensi

Division of Experimental Oncology D, Istituto Nazionale per lo Studio e la Cura dei Tumori, Via Venezian 1, 20133 Milano, Italy
1Ludwig Institute, 74 Avenue Hippocrate, 1200 Brussel, Belgium

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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 antigens 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) 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 antigens 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.

(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.

Correspondence to M. Sensi

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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-nitosoguanidine 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 of 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 V\textalpha chain transcripts.

In the present study we sought to determine nucleotide sequences encoding TCR \alpha and \beta chains from all available CTL clones in order to compare junctional TCRAV-AJ and TCRBV-BD-BJ regions encoding complementarity determining region (CDR) 3 and to identify the functional \alpha chains.

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

<table>
<thead>
<tr>
<th>CTL clones</th>
<th>Datea</th>
<th>Antigen</th>
<th>Peptide</th>
<th>TCRAVb</th>
<th>TCRBVb</th>
<th>Ref</th>
</tr>
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<tr>
<td>26/331</td>
<td>3/16/1983</td>
<td>MAGE-1</td>
<td>SAYGEPRKL</td>
<td>ND</td>
<td>ND</td>
<td>13</td>
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<td>82/35</td>
<td>7/7/1984</td>
<td>MAGE-1</td>
<td>SAYGEPRKL</td>
<td>8, 9</td>
<td>5</td>
<td>13</td>
</tr>
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<td>81/12</td>
<td>3/17/1986</td>
<td>MAGE-1</td>
<td>SAYGEPRKL</td>
<td>2, 10</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>81/9</td>
<td>3/17/1986</td>
<td>MAGE-1</td>
<td>SAYGEPRKL</td>
<td>ND</td>
<td>ND</td>
<td>13</td>
</tr>
<tr>
<td>81/20</td>
<td>3/17/1986</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>BAGE</td>
<td>AARAVFLAL</td>
<td>8</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>82/1</td>
<td>7/7/1984</td>
<td>BAGE</td>
<td>AARAVFLAL</td>
<td>2, 3</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
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<td>7/7/1984</td>
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<td>AARAVFLAL</td>
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<td>13</td>
<td>14</td>
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<tr>
<td>82/86</td>
<td>7/7/1984</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.

\( ^a \) Date of blood sample.

\( ^b \) TCRAV and BV expression was detected by PCR analysis.

**Fig. 1.** TCR \( \alpha \) and \( \beta \) 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). TCRAV and TCRBV gene subfamilies were classified according to Wilson \textit{et al.} (21) and to Wei \textit{et al.} (22) respectively. Sequencing of TCRBV5 and TCRBV8 amplified fragments did not allow identification of subfamily members. TCRAJ segments were assigned according to Koop \textit{et al.} (19), and TCRBD, BJ and BC elements according to Toyonaga \textit{et al.} (23). The TCRAV and TCRBV CDR3 length are defined according to Moss and Bell (24). TCR joining segment residues contributing to CDR3 are underlined.
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/HLA-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 composition and specific for identical antigenic epitopes (3,6,17,18) has been already indicated 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 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.

In vivo persistence of CTL clones with identical TCR clonotype composition and specific for identical antigenic epitopes (3,6,17,18) has been already described in non-tumor systems. Our data are also in keeping with the observation that HLA-A1-restricted CTL clones with a finite specificity for variant MAGE-1 peptides identical to the one displayed by one CTL clone derived from patient MZ2 pre-vaccination PBL were present and, in that case, dominant in the post-vaccination PBL (10).

CTL clones 81/9, 81/12 and 81/20, all deriving from the same mixed lymphocyte–tumor cell culture, are probably in vitro replicants and express an identical TCRAV2S2J52C1/TCRBV8J2S1C2 transcript present in replicant clones 82/82 and 82/66 obtained from PBL taken 1 year later. These clones display a TCRBV8S2J15C1/TCRBV8J2S1C2 rearrangement in their TCRBV chain that differs at a single nucleotide substitution in the CDR3 (Fig. 1). However, the deduced CDR3 amino acid composition was identical (Fig. 2). TCRα sequences are composed by TCRAV8S1J45C1/TCRBV8J1S1C1 with a stop codon at the TCRα10/TCRαJ45 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 TCR α and β CDR3 loops of identical length (Fig. 2), suggests a selection for certain TCR elements in the MAGE-1/HLA-Cw*1601-restricted T cell response of this patient.

A single anti-BAGE/HLA-Cw*1601 CTL clone was obtained from PBL taken at the time of surgery (CTL clone 25/244, Table 1) and it showed a TCRBV8S2J15C1/TCRBV8J2S1C2 rearrangement (Fig. 1) that was not present in either of CTL clones 82/1 and 82/2 (the latter identical to 82/66) obtained from PBL taken 1 year later. These clones display a TCRBV13S4J2S7C2 rearrangement in their TCRBV chain that differs at a single nucleotide substitution in the CDR3 (Fig. 1). However, the deduced CDR3 amino acid composition was identical (Fig. 2). TCRα sequences are composed by TCRAV8S1J45C1/TCRBV8J1S1C1 with a stop codon at the TCRα10/TCRαJ45 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 TCR α and β CDR3 loops of identical length (Fig. 2), 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...
The nucleotide sequence data reported in this paper are available from EMBL GenBank under accession nos X94088-X94097.

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Abbreviations

CDR complementarity determining region
CTL cytotoxic T lymphocytes
PBL peripheral blood lymphocytes

Note

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References