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Detection and Long-Term In Vivo Monitoring of Individual Tumor-Specific T Cell Clones in Patients with Metastatic Melanoma

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We investigated the presence of individual melanoma-specific T cell clones in patients with metastatic melanoma. Ten patients were examined for the presence of melanoma-reactive T cells using dendritic cells loaded with autologous tumor cells. Their specificity was tested using nonradioactive cytotoxicity test. Individual immunodominant T cell clones were identified by the clonotypic assay that combines in vitro cell culture, immunomagnetic sorting of activated IFN-γ+ T cells, TCRβ locus-anchored RT-PCR, and clonotypic quantitative PCR. All patients had detectable melanoma-reactive T cells in vitro. Expanded melanoma-reactive T cells demonstrated specific cytotoxic effect against autologous tumor cells in vitro. Three patients experienced objective responses, and their clinical responses were closely associated with the in vivo expansion and long-term persistence of individual CD8+ T cell clones with frequencies of $10^{-6}$ to $10^{-3}$ of all circulating CD8+ T cells. Five patients with progressive disease experienced no or temporary presence of circulating melanoma-reactive T cell clones. Thus, circulating immunodominant CD8+ T cell clones closely correlate with clinical outcome in patients with metastatic melanoma. The Journal of Immunology, 2007, 178: 6789–6795.
TABLE I. Characteristics of patients with metastatic melanoma

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex</th>
<th>Age at Diagnosis (years)</th>
<th>No. of Metastatic Sites</th>
<th>No. of Therapeutic Regimensa</th>
<th>Treatment Response/Survival (mo)</th>
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<tr>
<td>1</td>
<td>M</td>
<td>46</td>
<td>1</td>
<td>1 (AI)</td>
<td>sd/60</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>67</td>
<td>2</td>
<td>2 (D;A)</td>
<td>PR/22</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>58</td>
<td>3</td>
<td>1 (A)</td>
<td>PR/27</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>76</td>
<td>2</td>
<td>1 (A)</td>
<td>PD/12-died</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>64</td>
<td>5</td>
<td>2 (D;A)</td>
<td>PD/4-died</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>26</td>
<td>4</td>
<td>3 (D;AI;T)</td>
<td>PD/16-died</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>62</td>
<td>4</td>
<td>2 (TFCV;A)</td>
<td>PD/7-died</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>64</td>
<td>3</td>
<td>2 (A,F)</td>
<td>PD/8-died</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>54</td>
<td>1</td>
<td>2 (AI;F)</td>
<td>MR/14</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>70</td>
<td>3</td>
<td>3 (D;ALT)</td>
<td>PD/7</td>
</tr>
</tbody>
</table>

aTherapeutic regimens: dacarbazine (D); IFN-α2α (A) and/or IL-2 (I); temozolomide (T), fotemustine (F), cisplatin (C), vinblastine (V) in combination or temozolomide alone or fotemustine alone. M, Male; F, female; sd, stable disease; PR, partial response; PD, progressive disease; MR, mixed response.

Materials and Methods

Patient characteristics and cell collection

Ten consecutive patients with MM (American Joint Committee on Cancer stage IV) were enrolled in the study and treated according to the Czech Oncology Society guidelines with dacarbazine or temozolomide, fotemustin alone or fotemustin alone. I. All patients provided signed informed consent forms prepared in agreement with the Declaration of Helsinki and approved by the local Ethical Committee. As controls, PBMC from healthy individuals were used. Immature DC were obtained from histopathologically verified tumor tissue by cutting into small pieces (<2 mm), cultivated shortly before harvest, and frozen at ~80°C. PBMC were isolated using gradient centrifugation on Histopaque 1077 (Sigma-Aldrich) and either used immediately or stored deeply frozen at ~80°C.

Generation of melanoma-specific T cells

PBMC were incubated in 6-well culture plates in medium consisting of X-VIVO 10 (BioWhittaker) with 10% heat-inactivated human AB serum (Sigma-Aldrich), 80 U/ml DNase (Boehringer Mannheim), and 2 mM t-glutamine (Sigma-Aldrich) in an atmosphere of 5% CO2 for 2 h. The nonadherent fraction was used for the generation of CTLs. After removing the nonadherent fraction, adherent cells were then cultured in the presence of 800 U/ml GM-CSF (Schering-Plough) and 100 ng/ml IL-4 (Sigma-Aldrich). On days 3 and 6, half of the culture was replaced with fresh complete medium and cytokines were added. Immature DC were obtained with typical morphological and phenotypic characteristics (CD80+, CD86+, CD83low; data not shown). Melanoma cells were irradiated (60 Gy) and used as Ag pulsed to immature DC at a ratio of 1:1 on day 7. DC maturation was induced on day 8 using 1,000 U/ml TNF-α (Bender MedSystems). CTLs were elicited from autologous PBMC using stimulation by Ag-loaded DC. Washed nonadherent lymphocyte-rich PBMC fraction was resuspended in medium consisting of X-VIVO 10 (BioWhittaker) with 10% heat-inactivated AB serum (Sigma-Aldrich). Ag-specific T cell priming was initiated by Ag-pulsed mature autologous DC added to primary cultures at a ratio (T cell:DC) of 20:1. Cells were incubated in culture flasks at 37°C in 5% CO2 for 7 days, and the restimulation with the same Ag-pulsed DC was performed in a ratio (T cell:DC) of 2:1 to obtain the highest yield of tumor-reactive T cells. Activated IFN-γ-producing tumor-reactive T cells were harvested 24 h after restimulation using the Secretion Assay Cell Enrichment and Detection Kit (MACS Reagents; Miltenyi Biotec) as described previously (16).

Flow cytometric analysis

Cells were washed with PBS and incubated with FITC-conjugated, PE-conjugated, and allophycocyanin-conjugated Abs for 15 min on ice. Cell surface phenotype was analyzed by flow cytometry using anti-CD4-FITC, anti-CD8-FITC, anti-CD3-PE (Immunotech), and anti-IFN-γ-PE mAbs (Miltenyi Biotec). After washing with cold PBS, cells were fixed with 1%
blocking was performed by using 10 and the ARH-77 myeloma cell line served as negative controls. HLA class I Ab is shown in B. In patient no. 3, single immunodominant CD8\(^+\) T cell clones (Clone 3A) demonstrated specific cytotoxicity to autologous melanoma cells but not to allogeneic melanoma cells from patient no. 1. Negligible response against autologous DCs (DC only) and no response to irrelevant Ags such as myeloma cell line (ARH77) and allogeneic PBMC (AlloPBMC) were noted. Abrogation of CTL activity by anti-human HLA class I Ab is shown in B.

Identification of TCR\(\beta\) CDR3 sequences

Clonotypic assay (14, 15) was used for identification of individual melanoma-specific T cell clones based on the analysis of unique DNA sequences of TCR\(\beta\) CDR3. It was performed on sorted populations of activated or CD8\(^+\) melanoma-reactive T cells that were further separated immunomagnetically to CD3\(^+\)CD4\(^+\) and CD3\(^+\)CD4\(^-\) fraction by CD4\(^+\) selection (Miltenyi Biotech). At least 97% of the CD3\(^+\)CD4\(^+\) fraction were CD8\(^+\) T cells (data not shown). A total of 1 \times 10^5 IFN-\(\gamma\)-responsive T cells (either CD4\(^+\) or CD8\(^+\)) was used for mRNA extraction (Oligotex Direct mRNA Mini Kit; Qiagen). Anchored RT-PCR was performed using a modified version of the SMART method (SMART Race cDNA Amplification Kit; BD Clontech) as described previously (15). TCR\(\beta\)C primer (5'-GCTTCTGATGGCTCAAACACAGCGACCTC-3') was used to obtain TCR\(\beta\)PCR products from the 5' end to the start of the TCR\(\beta\) CDR3 region. The PCR product was ligated into the pGenT-Easy vector (Promega) and used for Escherichia coli transformation. Clones were defined by the presence of at least two identical DNA sequences (clonotypes) of the TCR\(\beta\) CDR3. For each PCR product, at least 50 colonies were selected, amplified by PCR with vector-specific primers, and used for direct sequencing to obtain TCR\(\beta\) CDR3 sequences corresponding to sorted, activated melanoma-reactive T cells.

In vivo quantitative monitoring of individual immunodominant T cell clones

The most immunodominant T cell clones were selected based on the frequency of their unique DNA sequence of the TCR\(\beta\) CDR3 region (>10% of the same clonotype among all bacterial colonies sequenced). Clone-specific primers and probes were designed for the TCR\(\beta\) CDR3 with Primer Express version 1.5. Clonotypic quantitative PCR (qPCR) was performed using an ABI PRISM 7700 on PBMC lysates or biopsy DNA as described previously (15). qPCR was a 50-cycle 2-step reaction using Platinum Taq (Invitrogen Life Technologies), with the annealing/extension phase at 65°C. A standard curve was plotted and template copies were calculated. Plasmid template for the qPCR standards was the same as that used in the sequencing of the CDR3. Each primer/probe set was tested against the target plasmids for the individual’s other primer/probe sets, and redesigned if necessary to ensure no cross-reactivity. Each set was also tested with PBMC derived from five different healthy donors, and we found no amplification with any of the 12 primer/probe sets. The high specificity and sensitivity (1 in 100,000 cells) of clonotypic qPCR has been shown previously (17, 18). Samples were analyzed in triplicate.
Results

Patients with MM have circulating melanoma-reactive CD4+ and CD8+ T cells

DC loaded with irradiated autologous tumor cells were used for activation of T lymphocytes in 10 patients with MM. Patient characteristics are shown in Table I. After two rounds of stimulation, low frequencies of IFN-γ-producing CD4+ (0.9–3.2%, median 1.3%) and CD8+ (0.6–2.4%, median 1.0%) T cells were detected (Fig. 1A). Melanoma-reactive IFN-γ-producing T cells were immunomagnetically separated. The percentage of activated CD3+ CD8+ T cells before magnetic separation was 1.8–4.4% (median 2.4%) and after the procedure reached 59.7–88.4% (median 76.2%) of all CD3+ T cells (Fig. 1B). Sorted CD3+ IFN-γ+ T cells (0.52–2.39 × 10^6 cells) were further expanded for 3–4 wk until at least 10^8 viable T cells were reached. After the expansion, 96.7–99.5% (median 99.0%) of cells were CD3+ T cells with a prevalence of 72.8–86.4% (median 81.1%) CD3+ CD8+ cells, remaining T cells were CD4+.

Expanded melanoma-reactive CTLs demonstrate specific cytotoxic potential

A specificity of expanded melanoma-reactive CTLs was tested against the original autologous melanoma cells in five patients (nos. 1, 3, 4, 7, and 8) with sufficient numbers of remaining tumor cells. Unselected T cells showed specific cytotoxicity to autologous melanoma cells. The percentage of activated CD8+ T cells (0.52–2.39 × 10^6 cells) were further expanded for 3–4 wk until at least 10^8 viable T cells were reached. After the expansion, 96.7–99.5% (median 99.0%) of cells were CD3+ T cells with a prevalence of 72.8–86.4% (median 81.1%) CD3+ CD8+ cells, remaining T cells were CD4+.

Identification of immunodominant T cell clones in vitro

We next analyzed the clonal composition of the sorted IFN-γ+ melanoma-reactive T cells and defined them by the unique sequences of their TCRβ CDR3 region in patients nos. 1–8. TCRβ loci of the sorted cells were amplified by template switch-anchored RT-PCR, as described in Materials and Methods, to ensure that all melanoma-reactive TCRβ sequences were amplified without bias to particular TCRβ V families. Thus, the melanoma-reactive clonotypes were represented in the anchored RT-PCR product with a relative frequency reflecting that found in the original sorted cell population. Sequencing of 50 bacterial colonies containing TCRB CDR3 from each sorted T cell clone was performed to identify the number and frequency of individual T cell clones in each sorted population. We have previously demonstrated the specificity and sensitivity of this approach to analyze the clonality of HIV-, GHV-, and GLV-specific T cells (15, 17). We have also demonstrated that only highly immunodominant T cell clones (forming at least 10% of all in vitro-identified clonotypes) are also detectable in vivo (15, 17).

Table II. Characteristics of immunodominant T cell clones

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Clone</th>
<th>Clone frequency</th>
<th>Amino acid sequence of CDR3 region</th>
<th>Amino acid sequence of TCRβ J region</th>
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<tr>
<td>1</td>
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<td>21/54</td>
<td>EAGRY</td>
<td>J1-4</td>
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<tr>
<td></td>
<td>1B</td>
<td>6/54</td>
<td>GHEHQN</td>
<td>J2-7</td>
</tr>
<tr>
<td>2</td>
<td>2A</td>
<td>24/51</td>
<td>LKTPSYIN</td>
<td>J1-1</td>
</tr>
<tr>
<td></td>
<td>2B</td>
<td>13/51</td>
<td>DTRKSSP</td>
<td>J1-3</td>
</tr>
<tr>
<td></td>
<td>2C</td>
<td>9/51</td>
<td>QAGTTD</td>
<td>J2-1</td>
</tr>
<tr>
<td>3</td>
<td>3A</td>
<td>26/50</td>
<td>DTSGIRPG</td>
<td>J2-2</td>
</tr>
<tr>
<td></td>
<td>3B</td>
<td>6/51</td>
<td>EAFLYNLSGJ</td>
<td>J1-5</td>
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<tr>
<td>4</td>
<td>4A</td>
<td>9/55</td>
<td>V4-1</td>
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<td>J1-1</td>
</tr>
<tr>
<td></td>
<td>4C</td>
<td>6/51</td>
<td>V5-1</td>
<td>J2-1</td>
</tr>
<tr>
<td></td>
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<td>6/51</td>
<td>V5-1</td>
<td>J2-1</td>
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<td>7/51</td>
<td>V17</td>
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<td>J2-6</td>
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<td>J1-4</td>
</tr>
<tr>
<td></td>
<td>6C</td>
<td>6/52</td>
<td>V11-2</td>
<td>J1-4</td>
</tr>
<tr>
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<td></td>
<td>7B</td>
<td>8/51</td>
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<tr>
<td></td>
<td>8B</td>
<td>7/51</td>
<td>PSSYIF</td>
<td>J2-5</td>
</tr>
</tbody>
</table>

Specific T cells (15, 17). We have also demonstrated that only highly immunodominant T cell clones (forming at least 10% of all in vitro-identified clonotypes) are also detectable in vivo (15, 17). Table II shows the number of successfully sequenced bacterial colonies and the number of different melanoma-reactive T cell clones identified for each patient. The frequency and amino acid sequences of CDR3 regions that occurred in >10% of bacterial colonies in vitro are also shown. These are likely to represent the most “dominant” T cell clones and their presence in peripheral blood of melanoma patients were further studied in vivo. We observed considerable heterogeneity in the clonality of melanoma-reactive T cell clones among the patients. Notably, melanoma-reactive CD8+ T cells from patients nos. 4–8 who died due to rapid cancer progression were highly polyclonal, comprising between 28 and 35 (median 31) individual clonotypes, whereas melanoma-reactive CD8+ T cells from patients with at least some measurable clinical response were far more oligoclonal, comprising between 5 and 22 (median 9) individual clonotypes. Indeed, within these oligoclonal repertoires, only one or two CD8+ T cell clones stood out as being clearly dominant in frequency for each patient with at least some treatment response. In one case (patient no. 3), we were able to...
identify a single immunodominant CD8$^+$ T cell clone (Clone 3A, Table II) that represented 6 of 15 in vitro-expanded clones initiated from a single-cell culture. This particular clone was the same as the most immunodominant CD8$^+$ T cell clone identified from the culture expanded in vitro from 10$^5$ melanoma-reactive IFN-γ$^+$ T cells in a single well, and we were able to monitor this clone in vivo for 24 mo as described in the next section.

Melanoma-reactive CD4$^+$ T cells were rather polyclonal in all patients regardless of their clinical response, comprising between 15 and 32 individual clonotypes with no clearly dominant clones (Table II, top).

Long-term persistence of immunodominant CD8$^+$ T cell clones correlates with better clinical outcome

Having identified individual immunodominant CD8$^+$ T cell clones in vitro, we next quantified the dominant clones at multiple time points after the patient was diagnosed with MM in peripheral blood. We have also examined the presence of these clones in tumor biopsies performed at the time of diagnosis and, in some cases, at the time of further metastatic spread of melanoma. We designed clone-specific PCR primers and probes specific for the TCRβ CDR3 region of each dominant melanoma-reactive clone, the sequence of which had been detected in at least 10% of bacterial colonies from the anchored RT-PCR product (Table II, bottom)—a total of 18 individual clones. We performed clonotypic quantitative real-time PCR on each sample from each recipient. Because a single T cell contains one productively rearranged TCRβ locus of a particular sequence, the copy number detected in each PCR was directly equivalent to the absolute number of melanoma-reactive clone cells present in the sample. Fig. 3 shows the levels of each immunodominant clone in the peripheral blood of seven patients characterized and numbered as shown in Table I. Notably, subjects 1–3 who are long-term survivors of MM have demonstrated a long-term persistence of the most dominant CD8$^+$ T cell clones in vivo in their peripheral blood. In contrast, subjects 4–7 had only temporary or no (subject 8) detectable immunodominant CD8$^+$ T cell clones in their peripheral blood. Quantification of absolute numbers of melanoma-reactive CD8$^+$ T cell clones showed in general some variability reaching from the threshold levels of qPCR 0.000001–0.001 of CD8$^+$ T cells in peripheral blood. It revealed a long-term persistence with minimal or mild expansion in long-term survivors but disappeared shortly after diagnosis of MM in nonsurvivors.

Because we did not see any dominant (comprising >10% of identical TCRβ CDR3 in DNA sequenced bacterial colonies in vitro) CD4$^+$ T cell clones, we tested the presence of the two most frequent melanoma-reactive CD4$^+$ T cell clones in their peripheral blood. Quantification of absolute numbers of melanoma-reactive CD8$^+$ T cell clones showed in general some variability reaching from the threshold levels of qPCR 0.000001–0.001 of CD8$^+$ T cells in peripheral blood. It revealed a long-term persistence with minimal or mild expansion in long-term survivors but disappeared shortly after diagnosis of MM in nonsurvivors.

Because we did not see any dominant (comprising >10% of identical TCRβ CDR3 in DNA sequenced bacterial colonies in vitro) CD4$^+$ T cell clones, we tested the presence of the two most frequent melanoma-reactive CD4$^+$ T cell clones in vitro. 5 of 55 (9.1%, subject 2) and 5 of 52 (9.6%, subject 4) bacterial colonies. None of these two CD4$^+$ T cell clones were detected by the quantitative clone-specific PCR in vivo in the peripheral blood of any of the follow-up samples. Similarly, in the case of the two minor CD8$^+$ T cell clones detected in vitro with frequencies 5 of 54 (9.3%, subject 1) and 5 of 51 (9.8%, subject 2), none of these clones was detected in vivo.

FIGURE 3. Long-term in vivo monitoring of immunodominant T cell clones. Individual immunodominant melanoma-reactive T cell clones identified in vitro at the time of metastatic dissemination (time 0) were quantitatively monitored in vivo in the peripheral blood of patient nos. 1–8. The number of clonotypic T cells is expressed as a percentage of all CD3$^+$CD8$^+$ T cells at the time of examination. No clonotypic T cells were detected in the peripheral blood of patient no. 8. The detection threshold for the clonotypic assay was 10$^{-5}$%.
Finally, we examined the presence of immunodominant CD8+ clones in original tumor-infiltrated lymph node biopsies by the clone-specific PCR and demonstrated the presence of these clones in all survivors (subjects 1–3) but only in three of six nonsurvivors studied (in subjects 4, 6, and 7). Subjects 6 and 8 were rebiopsied for a new metastatic involvement (12 and 6 mo, respectively, after their initial lymph node biopsy), but none of their immunodominant CD8+ T cell clones were detected. Subjects 9 and 10 were not studied by the clone-specific qPCR.

Discussion
By using DCs loaded with irradiated autologous tumor cells, we were able to identify circulating melanoma-reactive T cells in patients with MM. After stimulation, Ag-reactive T cells activate and produce IFN-γ. Both populations of CD4+ and CD8+ melanoma-reactive T cells were present. Such cells can be enriched immunomagnetically and further expanded in vitro (16, 19). To generate melanoma-specific CTLs for further study, we expand IFN-γ+ melanoma-reactive T cells that contain most of the immunodominant T cell clones. Despite the advanced stage of tumor spread, it was possible to expand functional melanoma-reactive T cells. For the most part, CD8+ CTLs were expanded and demonstrated a tumor-specific cytototoxic effect. Cytotoxicity against autologous melanoma cells was blocked by an anti-HLA class I Ab, indicating that the effect was most likely mediated through CD8+ HLA class I interaction. This finding confirms an important role for CD8+ CTLs in specific cytotoxicity that is in agreement with studies using a defined melanoma-specific peptide HLA class I-restricted Ag (9, 10).

In this study we identified in vitro, and quantified in vivo, individual immunodominant melanoma-reactive CD8+ T cell clones by developing an approach that combines in vitro cell culture, immunomagnetic sorting of activated IFN-γ+ T cells, TCRβ locus-anchored RT-PCR, and clonotypic qPCR. In published studies, the identification of clonal T cell responses has been accomplished by flow cytometry using mAbs directed against TCRβ V regions, by CDR3 spectratyping, or by microplate hybridization assay. These studies were able to demonstrate that the TCRβ repertoire is highly variable; for example, monoclonal, oligoclonal, or polyclonal CTL responses can be induced depending on the tumor Ag used (20–22). Interestingly, either monoclonal or polyclonal responses can be elicited by the same peptide derived from a MAGE-3.A1 tumor-specific Ag in different individuals (21, 22). Yet, using these techniques does not enable detailed analysis of individual T cell clones because, in the case of flow cytometry, the series of Abs are not complete enough to analyze all TCRβ V region families; in the case of spectratyping and microplate hybridization assay, TCRβ V region families, but not individual T cell clones within these families, can be identified (23–25). Our approach obviated skewing of the clonotype population by prolonged stimulation and propagation. Furthermore, the anchored RT-PCR step used to amplify TCRβ CDR3 regions of all T cell clones present in the sorted population avoided the incomplete coverage of, and bias to, particular TCRβ V families conferred by using TCRβ V-specific PCR primers. This approach required no prior knowledge of either the specific target Ag or the MHC-restricting element, and when combined with qPCR, it allowed for the sensitive and specific quantification of melanoma-reactive T cell clones in the patient at any point and in any blood or tissue sample. In agreement with spectratyping and microplate hybridization studies (23–25), we were able to detect oligoclonal or monoclonal expansions of melanoma-reactive CD8+ T cell clones. The sensitivity of clone-specific qPCR reached the threshold of \(1 \times 10^{-6}\), thus allowing us to monitor low-frequency clones. It has been shown that the frequency of naive T cells recognizing melanoma-specific peptide Ags such as MAGE-A3, gp100, NA17, LAGE-1, or MAGE-A10 exists in vivo with the frequencies of \(1 \times 10^{-7}\) to \(1 \times 10^{-8}\) (10). The Melan-A/MART-1-T2R–36 is a remarkable exception with a very high naive T cell frequency of \(\sim 5 \times 10^{-4}\) of CD8+ T cells (26). Recently, Speiser et al. (27) demonstrated that human CD8+ T cell clones specific to the Melan-A/MART-1 persisted for >1 year in a patient with melanoma, reaching up to 2.5% of the circulating CD8+ T cells. At the time of the melanoma-specific T cell clonal expansion, the disease stabilized but subsequently progressed with loss of Melan-A-specific T cell clones (27). In agreement with that study, we were able to demonstrate that the immunodominant CD8+ T cell clones in five patients with progressive disease were only temporally present (or were undetectable in one case) in the circulation, and all of these patients subsequently died of their disease. Melanoma-reactive clones were also undetectable in tumor biopsies taken at the time of tumor progression shortly before death. In contrast, all three patients with objective responses had melanoma-reactive CD8+ T cells present in the circulation for 18–60 mo with frequencies of \(10^{-4}\) to \(10^{-3}\). These low-frequency CD8+ T cell clones can probably be expanded as a response to tumor growth or as a response to melanoma treatment. Such observation further confirms the importance of individual tumor-reactive CD8+ T cell clones in cancer survival. Thus, prolonged persistence of melanoma-reactive CD8+ T cell clones is associated with better survival.

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Disclosures
The authors have no financial conflict of interest.

References


