Oligoclonal T-Cell Receptor Usage of Melanocyte Differentiation Antigen-reactive T Cells in Stage IV Melanoma Patients

David Schrama, Mads Hald Andersen, Patrick Terheyden, Louise Schroder, Lars Østergaard Pedersen, Per thor Straten, and Jürgen C. Becker

Department of Dermatology, Julius Maximalius-University, 0-97080 Wurzburg, Germany [D. S., P. T., J. C. B.]; and Department of Tumor Cell Biology, Division of Cancer Biology, Danish Cancer Society, DK-2100 Copenhagen, Denmark [M. H. A., L. S., L. Ø. P., P. t. S., J. C. B.]

Introduction

Recent technological advances (i.e., the introduction of tetrameric MHC-peptide complexes, ELISPOT analysis, and TCR clonotype mapping) revealed new insights into T-cell responses against viruses and neoplastic cells (1–3). For the latter, it became obvious that specific T-cell responses are spontaneously present and, as in the case of melanoma, are frequently directed against self-antigens (4). Furthermore, the magnitude of TCR-repertoire usage in antitumor T-cell responses was larger than previously anticipated (5). Still, it should be noted that most studies using TCR-repertoire analyses are limited by the lack of knowledge about the antigen specificity of overexpressed T-cell clonotypes (6–8). However, in our study, we attempted to characterize the TCR-receptor repertoire of melanoma-reactive T cells identified by tetrameric MHC-peptide complexes (9). The technique used for TCR-repertoire analysis did only allow to distinguish between different TCR families but not between individual T-cell clones. Here, we tested PBLs of stage IV melanoma patients for reactivity against MDAs MART-1 and gp100, isolated such cells using specific MHC-peptide complexes coupled to magnetic beads, and subsequently established their TCR BV region usage and clonotype composition by DGGE clonotype mapping.

Materials and Methods

Ex vivo ELISPOT analysis of peripheral blood lymphocytes obtained from stage IV melanoma patients demonstrated reactivity against peptides derived from MART-1 and gp100. However, the number of reactive T cells was <1% of total lymphocytes as detected by flow cytometry using tetrameric MHC/peptide complexes. Despite this low frequency, we were able to directly isolate these populations ex vivo by means of magnetic beads coated with MHC/peptide complexes and to subject these cells to T-cell receptor clonotype mapping. This analysis revealed that the MART-1/A*0201- and gp100/A*0201-reactive T-cell populations are composed of oligoclonal T cells that engage several T-cell receptor β chain families. Longitudinal studies using this approach may result in a better correlation between T-cell reactivity and the course of neoplastic disease.

Received 10/30/00; accepted 11/27/00.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported by grants from the Danish Cancer Society, the Bundesministerium für Forschung und Bildung, EKSF-Würzburg Project B-12, the Danish Medical Council, the Novo Nordisk Foundation, and the Wilhelm-Sander-Stiftung. J. C. B. is a recipient of a Ph.D. sponsorship from the Danish Cancer Society.

2 To whom requests for reprints should be addressed, at Department of Dermatology, University of Würzburg, Josef-Schneider-Str. 2, Building 13, 0-97080 Würzburg, Germany. Fax: 49-931-201-2700; E-mail: becker-je.derm@medizin.uni-wuerzburg.de.

3 The abbreviations used are: TCR, T-cell receptor; BV, β variable; DGGE, denaturing gradient gel electrophoresis; TIL, tumor-infiltrating lymphocyte; PBL, peripheral blood lymphocyte; MDA, melanocyte differentiation antigen; RT-PCR, reverse transcription-PCR.
Results and Discussion

Several studies have focused on the complexity of TCR usage of tumor-reactive T cells (7, 8, 11). However, the value of these studies was limited by the scanty characterization of the specificity of the analyzed T cells. Therefore, we performed a tetramer-guided ex vivo analysis of the complete TCR-repertoire of MDA-recognizing T cells present in the peripheral blood of stage IV melanoma patients.

We first analyzed the ability of PBLs of the two stage IV melanoma patients to react against MDA (i.e., modified MART-126–35 and gp100209–217 epitopes) by measuring the specifically induced production of IFN-γ in the ELISPOT assay. As shown in Fig. 1, each spot represents a peptide-reactive, IFN-γ-producing T cell. The average number of spots per peptide was calculated using a CCD scanning device and a computer system. For patient 1, an average of 86 MART-1 peptide-specific spots per 3 × 105 cells could be detected (Fig. 1A). Patient 2 showed a strong response to the gp100-derived peptide with an average of 73.5 peptide-specific spots per 3 × 105 cells (Fig. 1B). This reactivity increased to 172 peptide-specific spots in the second sample obtained 5 months later (Fig. 1C).

Recently, it has been demonstrated that the ELISPOT assay underestimates the number of peptide-recognizing T cells (12). This is due to the fact that only effector cells that can produce IFN-γ on stimulation can be detected; thus, subpopulations such as naive, exhausted, or anergic cells are missed. Therefore, we determined the percentage of peptide-recognizing T cells among PBLs of the patients by flow cytometry using MART-1/A*0201- or gp100/A*0201-tetrameric complexes. For patient 1, 0.63% of all lymphocytes were stained with the MART-1/A*0201-tetrameric complex (Fig. 2A). The amount of gp100/A*0201-tetramer complex-detected T cells in the peripheral blood of patient 2 was 0.80% and 0.71%, respectively, in the two samples analyzed (Fig. 2, C and D). Due to the limited amount of blood available, we excluded the detailed characterization of tetramer-stained cells because we anticipated that large cell numbers would be needed for subsequent tetramer-guided isolation of MDA-recognizing T cells. Moreover, several excellent studies have already addressed this issue previously (9, 12–16).

Over the past few years, much effort has been spent on the clonal characterization of T cells recognizing melanoma-associated antigens, in general, and MDA, in particular. However, inconsistent and sometimes contradicting results were obtained. These were due, at least in part, to confounding factors inherent in the experimental strategy (e.g., the analysis of in vitro expanded T cells; Refs. 11 and 17), the restriction of the TCR analyses to only a fraction of the TCR BV repertoire (9) or the clonotype mapping of only overexpressed TCR BV families (7). To avoid these complicating factors, we characterized the clonal composition of MDA-recognizing T-cell populations directly, by ex vivo analyses. For this purpose, we applied tetramer-guided isolation in combination with RT-PCR/DGGE-based TCR clonotype mapping. The latter technique allows the analysis of the complete TCR BV repertoire for the presence of clonal expanded T cells both in overexpressed and normally expressed BV families, even when only small numbers of cells are available.

The first series of experiments addressed the feasibility of using specific MHC-peptide complexes to isolate reactive T cells. To this end, we coated magnetic beads with MART-1/A*0201-complexes and used these to isolate MART-1-reactive cells from a T-cell line that was obtained from TILs by in vitro expansion for several weeks in interleukin 2-containing medium. The frequency of MART-1-reactive cells was measured by peptide-induced production of IFN-γ in the ELISPOT assay before and after isolation. The assay was not performed immediately but 7 days after the isolation procedure to avoid the presence of contaminating MART-126–35 peptides in the negative controls. Before isolation, an average of 80 MART-1 peptide-specific spots per 105 cells could be detected (data not shown). After isolation, this number increased to >250 spots per 1.3 × 105 cells (data not shown). Thus, this method of isolation was, indeed, highly efficient to enrich specific peptide/MHC-reactive T cells, because afterward such cells were expressed at two logs greater frequency.

These encouraging results prompted us to use magnetic beads coated with either MART-1/A*0201- or gp100/A*0201-complexes to sort out T cells recognizing these antigens from PBLs ex vivo without any measures of prior in vitro expansion. The yield of reactive cells by this approach ranged between 0.02% and 0.09%. Although these numbers correspond well with the number of T cells detected with fluorescent peptide/A*0201-tetrameric complexes by flow cytometry,
visual inspection of isolated cells revealed that some of them were labeled only with one bead. Consequently, we increased the specificity of the isolation procedure by additional washing steps so that only lymphocytes with several bound peptide/A*0201-coated beads were seen (Fig. 2B). However, these additional washing steps reduced the cellular yield to 0.008–0.023%. Immediately after isolation, the T cells were subjected to TCR clonotype mapping, which revealed their oligoclonal origin. T cells of patient 1 recognizing MART-1/A*0201 were composed of 11 clones that belonged to six different TCR BV families (i.e., 5, 6, 12, 14, 21, and 22; Fig. 3). For patient 2, T cells recognizing gp100/A*0201 included a higher number of clones, namely 19 for the first and 20 for the second time point analyzed; these T cell clones belonged to eight or nine TCR BV families, respectively (data not shown). Hence, the number of clonally expanded T cells detected among MDA-specific T cells in the peripheral blood of melanoma patients is substantially lower than the number of clonally expanded cells present in TILs comprising approximately 40–60 clonotypes spanning almost all TCR BV families (5). However, it is important to note that the additional washing steps in the isolation process might have removed some low-affinity clones. Nevertheless, the extent of the TCR-repertoire usage in the circulating T-cell population reactive against only one MDA suggests a rather tight selection of T-cell clonotypes for recruitment into the pool of TILs. This notion is based on the assumption that if the magnitude of the antigenic repertoire of melanoma as well as the number of T-cell clonotypes recognizing one individual peptide/HLA-complex are used to predict the amount of TCR BV usage among TILs, this hypothetical number would be considerably larger than what has been detected experimentally (5, 18).

The comparison of T-cell clonotypes present in different metastatic melanoma lesions from the same individual revealed that matching clonotypes were generally not detectable in more than one lesion (5). This observation indicated the predominance of localized T-cell expansions at least for the effector phase of immune responses to melanoma. The presence of MDA-reactive CD8+ T-cell clonotypes in the peripheral blood of stage IV melanoma patients raises the question why such cells are not part of the TIL repertoire and, hence, resulting in identical T-cell clonotypes being present in several metastatic sites. A possible reason for these discrepant observations may be that the analysis of peripheral blood provides only a momentary glimpse of ongoing immune responses that do not necessarily reflect the complexity and heterogeneity of a dynamic T-cell response. This concept prompted us to test whether the clonotypes composing the MDA-reactive T-cell population would change over time or whether this composition is durable. Remarkably, comparative RT-PCR/DGGE analysis of circulating gp100/A*0201-recognizing T cells from patient 2, isolated 5 months apart, revealed that some of the clonotypes detected (e.g., within the BV families 12 and 17) resolved at positions in the gel which indicated identity (Fig. 4), a notion unequivocally proven by subsequent sequencing of the CDR3 region (data not shown). Therefore, it is justified to conclude that the T-cell reactivity against MDA in peripheral blood is maintained by a finite and persistent repertoire of clonally expanded T cells. However, these T-cell clonotypes, particularly the persisting ones, seem to have only a limited capacity to infiltrate the tumor microenvironment (19). This conclusion is in line with several clinical studies demonstrating a lack of correlation between T-cell reactivity measured in peripheral blood and the course of neoplastic disease (20).

In summary, we demonstrate the magnitude and complexity of cellular immune responses to defined MDA-derived T-cell epitopes in peripheral blood of melanoma patients. These findings emphasize the need to analyze not only the peripheral blood but also the tumor site, as well as secondary and tertiary lymphatic tissues, for the presence and activation status of tumor-reactive T cells to obtain more adequate insights into immune responses to solid tumors.

Acknowledgments

We thank Eva Fuchs, Tina Seremet, and Claudia Siedel for excellent technical assistance. J. C. B. extends special thanks to Eva-B. Bröcker and Jes Forchhammer for ongoing support and encouragement.

References

Oligoclonal T-Cell Receptor Usage of Melanocyte Differentiation Antigen-reactive T Cells in Stage IV Melanoma Patients

David Schrama, Mads Hald Andersen, Patrick Terheyden, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/61/2/493

Cited articles
This article cites 20 articles, 7 of which you can access for free at:
http://cancerres.aacrjournals.org/content/61/2/493.full#ref-list-1

Citing articles
This article has been cited by 5 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/61/2/493.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://cancerres.aacrjournals.org/content/61/2/493.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.