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*J Immunol* 2006; 177:8212-8218; doi: 10.4049/jimmunol.177.11.8212

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Spontaneous CD8 T Cell Responses against the Melanocyte Differentiation Antigen RAB38/NY-MEL-1 in Melanoma Patients

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The melanocyte differentiation Ag RAB38/NY-MEL-1 was identified by serological expression cloning (SEREX) and is expressed in the vast majority of melanoma lesions. The immunogenicity of RAB38/NY-MEL-1 has been corroborated previously by the frequent occurrence of specific Ab responses in melanoma patients. To elucidate potential CD8 T cell responses, we applied in vitro sensitization with overlapping peptides spanning the RAB38/NY-MEL-1 protein sequence and the reverse immunology approach. The identified peptide RAB38/NY-MEL-150–58 exhibited a marked response in ELISPOT assays after in vitro sensitization of CD8 T cells from HLA-A*0201+ melanoma patients. In vitro digestion assays using purified proteasomes provided evidence of natural processing of RAB38/NY-MEL-150–58 peptide. Accordingly, monoclonal RAB38/NY-MEL-150–58-specific T cell populations were capable of specifically recognizing HLA-A2+ melanoma cell lines expressing RAB38/NY-MEL-1. Applying fluorescent HLA-A2/RAB38/NY-MEL-150–58 multimeric constructs, we were able to document a spontaneously developed memory/effector CD8 T cell response against this peptide in a melanoma patient. To elucidate the Ag-processing pathway, we demonstrate that RAB38/NY-MEL-150–58 is produced efficiently by the standard proteasome and the immunoproteasome. In addition to the identification of a RAB38/NY-MEL-1-derived immunogenic CD8 T cell epitope, this study is instrumental for both the onset and monitoring of future RAB38/NY-MEL-1-based vaccination trials. The Journal of Immunology, 2006, 177: 8212–8218.

Critical advances have been made toward understanding the magnitude and characteristics of cellular immune responses in cancer patients (for review, see Refs. 1 and 2). In particular, the discovery of tumor Ags and the precise characterization of their immunogenic sequences involved in T cell mediated anti-tumor immunity (3) have allowed, for the first time, to systematically analyze anti-tumor T cell responses and have substantially progressed the development of specific cancer vaccines (4–6). Vaccination trials are focused mainly on the use of immunogenic epitopes derived from the two major groups of nonmutated shared Ags, namely cancer-testis (i.e., NY-ESO-1, MAGE, and SSX) and differentiation Ags (i.e., Melan-A, tyrosinase, and NY-Br1). Melanocyte differentiation Ags are expressed in normal melanocytes and melanomas, and their function in melanogenesis is frequently associated with pigment production. There is considerable evidence that melanocyte differentiation Ags presented to the immune system of melanoma patients can overcome tolerance and induce T cell-based immunity (7–9). The relative immunogenicity of Melan-A/MART-1 is thought to be the highest of this group of Ags. We could demonstrate previously that Melan-A/MART-1-specific CD8 T cells with potential tumoricidal capacity are selectively primed in vivo and are directly detectable by fluorescent HLA-A2/peptide multimeric constructs (thereafter multimers) even in circulating lymphocytes from melanoma patients (10). Apart from the Melan-A/MART-1-specific CD8 T cell repertoire, but similarly to the apparent paucity of ex vivo detectable CD8 T cell responses to cancer tests Ags, multimers can only rarely be used to readily identify CD8 T cells specific for other differentiation Ags, e.g., tyrosinase (11, 12). Notably, the application of CD8 T cell epitopes derived from melanocyte differentiation Ags in cancer vaccination trials has demonstrated favorable effects on disease progression in individual patients with only very limited toxicity (13–15). Recently, we reported on the melanocyte differentiation Ag RAB38/NY-MEL-1 (thereafter RAB38) (16), that is highly expressed in normal melanocytes and melanoma tissues but not in other normal tissues or cancer types. Importantly, RAB38 is immunogenic leading to spontaneous Ab responses in a large proportion of melanoma patients (17). In this study, we demonstrate for the first time the identification of a naturally occurring RAB38-specific CD8 T cell response through the association of in
vitro sensitization with overlapping peptides and in silico prediction of potential HLA class I ligands. Tumor cell recognition and in vitro proteosomal digestion assays document natural processing and presentation of the HLA-A2 restricted RAB38_{40-58} peptide. We show that, in the T cell repertoire of melanoma patients, RAB38_{40-58}-specific CD8 T cells exist that can even be detected spontaneously applying multimer technology and are endowed with sufficient avidity to lyse RAB38-expressing tumor cells. Its in vivo immunogenicity reflected by the presence of cellular and humoral anti-RAB38 immune responses in cancer patients opens the avenue for developing Ag-specific immunotherapy targeting RAB38.

**Materials and Methods**

**PBMCs, tumor cell lines, and EBV-transformed B-LCL cells**

Peripheral blood samples were obtained from HLA-A^2^+ patients with stage III/IV malignant melanoma and from 11 HLA-A^2^+ and 10 HLA-A^2^+ healthy donors after written informed consent. Mononuclear cells were purified and immediately frozen as described (18). All used HLA-A^2^+ tumor cell lines, and EBV-transformed B-LCL cells exhibited the A^0201 subtype.

**Flow cytometry immunofluorescence analysis and peptides**

PE-labeled HLA-A2/petide multimers were synthesized around RAB38_{40-58} (VLHWDPEVTE) or Flu-MAMA-s6 (GILGFVFTL), a peptide from the influenza matrix protein. Enriched CD8 T cells (>80%) were stained with the appropriate PE-labeled multimers and mAbs as described previously (18). Cells were immediately analyzed on a FACS Calibur using CellQuestPro software, both from BD Biosciences. mAbs also were from BD Biosciences. Peptides were synthesized by standard solid-phase chemistry on a multiple peptide synthesizer (Biosynthesis). Recognition of HEK293 EBNA cells by RAB3850–58-specific T cells was tested in an MTT colorimetric assay using the intracellular IFN-γ assay.

**RNA extraction, quantitative real-time PCR, and Western blot analysis**

RNA extraction, RAB38-specific quantitative real-time PCR, and Western blot assays to detect circulating RAB38-specific Ab responses were performed as described previously (17). To analyze the proteasome subunits, rabbit anti-hLMP-2 (b1i; PW8345), rabbit anti-hLMP-7 (b5i; PW8355), rabbit anti-hMECL-2 (b2i; PW8350), rabbit anti-hb5 (PW8895), mouse anti-hh1 (Y; PW8140), and mouse anti-hb2 (Z; PW8145) were purchased from Affiniti Research Products. Secondary HRP-labeled goat anti-rabbit Abs or anti-mouse (Jackson ImmunoResearch Laboratories) were used, respectively. Detection of specific binding was performed with the ECL detection kit (Amersham Biosciences).

**Results**

RAB38-specific CD8 T cell responses in circulating lymphocytes from HLA-A2 melanoma patients

Initially, a HLA-A^0201^ melanoma patient (ZH-48) with RAB38-expressing tumor lesions and a spontaneous anti-RAB38 Ab response was selected to elucidate CD8 T cell responses against RAB38. Total CD8 T cells were stimulated with 18-aa-long peptides spanning the entire RAB38 protein sequence and overlapping by 10 amino acids, in the presence of autologous APCs. Cultures were tested for the presence of specific, IFN-γ-secreting CD8 T cells by ELISPOT assays using those peptides. A specific response to the RAB38_{41-58} peptide corresponding to a frequency of 2.1% of IFN-γ-secreting CD8 T cells was clearly detected (Fig. 1a). In addition, this patient exhibited a weaker, still significant response (0.2%) against RAB_{105-122} peptide (data not shown). No clear response was detectable against all the other peptides, although for some of the tested peptides, the proportion of IFN-γ-secreting CD8 T cells was 2- to 3-fold higher in the presence than in the absence of the corresponding peptide (data not shown).

**FIGURE 1.** Enumeration of RAB38_{40-58}-specific CD8 T cells in melanoma patients and isolation of specific monoclonal populations. a and b, IFN-γ ELISPOT analysis of PBMCs from patient ZH-48 and ZH-104 stimulated with RAB38_{41-58} and RAB38_{50-58} peptides, respectively, in the absence or presence of relevant peptides. Numbers correspond to the percentage of specifically IFN-γ-secreting CD8 T cells. c, IFN-γ secretion assay of PBMCs from patient ZH-104. After in vitro sensitization, PBMCs were stimulated in the absence or presence of RAB38_{50-58} peptide and stained with a cytokine secretion detection kit. FACS single-cell sorting was used to isolate IFN-γ+ CD8 T cells and consequently to generate clonal populations. Numbers correspond to the percentage of specifically IFN-γ-secreting CD8 T cells. d, Clone ZH-104/7 was stained with PE-labeled A2/RAB38_{40-58} (left) or A2/FLU-MAMA_{49-66} (right) multimers, respectively, anti-CD8, and anti-CD3 Abs, and analyzed by flow cytometry. Dot plots are gated on CD3+ lymphocytes.
To further restrict the number of candidate epitopes, RAB3841–58 sequence was screened for peptides predicted to bind to HLA-A2 molecules with high affinity (i.e., with a score of >20) by the SYFPEITHI algorithm. As a result, we chose the following two peptides for analysis: RAB3841–50 (TIGVDFALKV; score 21) and RAB3848–50 (VLHWDPETV; score 23). Using those two peptides, CD8 T cells of four melanoma patients were stimulated with one round of in vitro sensitization, and cultures were tested in ELISPOT assays. Unfortunately, PBMCs from patient ZH-48 were no longer available as the patient died from disease progression. Although no reactivity was detected against the RAB3850–58 peptide, RAB3850–58 peptide induced significant responses with values of IFN-γ-secreting CD8 T cells being at least 3-fold higher than baseline (irrelevant peptide) in three (ZH-104, ZH-169, ZH-224) of four patients. In these patients, percentages of IFN-γ-secreting CD8 T cells varied between 0.35 and 0.9% (data not shown). Of these patients, one patient (ZH-104) exhibited a detectable anti-RAB38 Ab response (data not shown). PBMCs of patient ZH-104 with a frequency of 0.35% of IFN-γ-secreting CD8 T cells (Fig. 1b) were further used to perform an additional round of restimulation. After reaching a frequency of 2.1%, IFN-γ-secreting CD8 T cells were isolated from the cultures using a cytokine secretion detection kit and FACS sorting (Fig. 1c). This procedure resulted in the isolation of monoclonal T cell populations that were specifically stained by HLA-A*0201 multimers folded around the RAB3850–58 peptide, but not by irrelevant HLA-A2/Flu58–66 multimers (Fig. 1d). Clone ZH-104/7 is shown as a representative example.

**Functional analysis of RAB3850–58-specific CD8 T cell clones**

First, we confirmed the specificity of the generated CTL clones. In a chromium release cytotoxicity assay, the clone ZH-104/7 lysed T2 cells incubated with peptide RAB3850–58 (with a functional avidity of 10–10 M) but not T2 cells incubated with the irrelevant Flu58–66 peptide (Fig. 2a). To further document HLA-A2-restricted recognition, we assessed the ability of RAB3850–58-specific CTL clones to lyse RAB3850–58 peptide-pulsed EBV-transformed B-LCL cells expressing or not HLA-A2. As depicted in Fig. 2b, only HLA-A*0201+ EBV-transformed B-LCL cells were recognized. Fine specificity of Ag recognition was assessed using RAB3850–58 nonameric peptide, and, additionally, the N- and C-terminally extended RAB3850–58 and RAB3850–59 decameric peptides, respectively. Peptide titration experiments showed clearly that the RAB3850–58 decameric peptide was as efficiently recognized as the nonamer, while the relative antigenic activity of the C-terminal extended RAB3850–59 decameric peptide was at least 2 orders of magnitude lower than the RAB3850–58 nonameric peptide (Fig. 2a). Among the human RAB family, RAB38 is closest related to RAB32 both at the DNA and protein levels. RAB32 has been shown to be ubiquitously expressed in multiple normal tissues (23). To exclude reactivity against peptides derived from the RAB32 sequence, recognition of clone ZH-104/7 was tested in a chromium release assay using T2 cells pulsed with the RAB3241–59 peptide exhibiting the highest sequence homology to RAB3850–58 (~60%). Importantly, no cross-recognition to this peptide was observed (Fig. 2a).

RAB3850–58 peptide is naturally processed and presented

In addition to sufficient binding capacities to HLA class I molecules, an important requirement for a peptide to be naturally presented as CD8 T cell epitope is proper excision from the protein by the proteolytic machinery. Therefore, we analyzed in vitro proteasome-mediated digestions of a 20-mer polypeptide (RAB3844–63) encompassing the RAB3850–58 peptide. The 20-mer was incubated for different time periods with standard proteasomes purified from human erythrocytes as reported (24). Applying the CTL clone ZH-104/7 that specifically recognizes both RAB3850–58 and RAB3850–59 epitopes, the digested products were tested for recognition when pulsed onto T2 cells. As demonstrated in Fig. 3a, the 15-, 30-, and 60-min digests obtained with the proteasomes were recognized by the CTL, whereas no recognition was observed for the RAB3844–63 polypeptide before digestion (0 min). This suggests that, indeed, the CTL clone could recognize a product generated by the proteasome in vitro, most likely due to an efficient cleavage at the C terminus. To assess whether RAB3850–58-specific T cells also were able to recognize endogenously produced HLA-A2/RAB3850–58 complexes, COS-7 cells were transfected transiently with plasmids encoding HLA-A2 and either RAB38 or Melan-A as a control. Transfected COS-7 cells were tested for their capacity to induce TNF-α secretion by RAB3850–58-specific clone ZH-104/7. As shown in Fig. 3b, the CTL clone produced high levels of TNF-α in the presence of cells cotransfected with plasmids encoding HLA-A2 and RAB38 but not HLA-A2 and Melan-A. Untransfected cells or cells expressing either RAB38 or HLA-A2 were not recognized (data not shown). Melan-A26–35-specific clone ZH-7/5 served as a positive control. Similarly, RAB3850–58-specific clone ZH-104/7 was able to specifically secrete IFN-γ when cocultivated with HLA-A2-expressing HEK293 EBNA cells that were transiently transfected with RAB38 plasmid (Fig. 3c). To document T cell recognition of tumor cells endogenously expressing RAB38, two HLA-A2+ melanoma cell lines, NW-Mel-16 and NW-Mel-449, were tested for recognition by the RAB3850–58-specific T cell clone ZH-104/7. As already demonstrated previously by Northern blotting (16), we confirmed a

**FIGURE 2.** Functional characterization of RAB3850–58-specific clones. a. Lytic activity of clone ZH104/7 against T2 cells was tested in a 51Cr release assay at an E:T ratio of 10:1 in the presence of exogenously added RAB3850–58 (●), RAB3850–59 (●), RAB3850–58 (△), RAB3251–59 (□), and FLU-MA58–66 peptide (○). b. Lytic activity of the clone was tested in a 51Cr release assay against EBV-transformed B-LCL cells expressing or not HLA-A2 in the presence of RAB3850–58 or FLU-MA58–66 peptide (data not shown). Concentrations of peptides corresponded to 1 μM, E:T ratio = 10:1.
weaker expression of RAB38 in those melanoma cell lines, compared with melanocytes using quantitative real-time PCR (Fig. 3d and Ref. 17). Protein expression was further confirmed by Western blot analysis (data not shown). As shown in Fig. 3e, RAB38 "HLA-A2" NW-Mel-16 tumor cell line was efficiently lysed by the CTL clone, both in the absence and presence of exogenously added peptide. Similar results were obtained for tumor cell line NW-Mel449 (data not shown). RAB38 "HLA-A2" MCF-7 cells were not lysed unless peptide RAB3850–58 was added exogenously. Finally, RAB38 "HLA-A2" NW-Mel-1453 was not lysed by the CTL clone, neither in the presence nor in the absence of synthetic peptide. Lysis of RAB38 "HLA-A2" NW-Mel-16 cells by RAB3850–58-specific CTL could be blocked using w6/32 mAb (data not shown). Similar results, in terms of peptide recognition as well as recognition of transfected cells and tumor cells, were obtained with six other CTL clones (ZH-104/1, ZH-104/19, ZH-104/23, ZH-104/24, ZH-104/28, and ZH-104/30) from patient ZH-104. Altogether, these data identify RAB3850–58 as an HLA-A2-restricted CD8 T cell epitope that is correctly processed by the intracellular machinery of the tumor cells and presented on the cell surface of melanoma cell lines.

**RAB3850–58 peptide is processed by the immunoproteasome**

Proteasomes play a critical role in the generation of antigenic peptides for MHC class I presentation. As shown above, in vitro digestion analysis and specific T cell recognition of NW-MEL-16 and NW-MEL-449 cells expressing both RAB38 and the standard proteasome strongly indicate efficient processing of RAB3850–58 peptide by the multicatalytic protease complex of the tumor cells. However, mature dendritic cells (DCs) and tumor cells located in an IFN-γ-rich environment constitutively express the immunoproteasome that may significantly differ in the catalytic activities and, consequently, in the cleavage specificities (25, 26). Thus, we aimed to determine the relative efficiency of the two proteasome types to process the RAB3850–58 peptide. To this end, the NW-Mel-16 tumor cell line was treated with 100 U/ml IFN-γ for 2, 4, 6, and 10 days before being tested for recognition by the RAB3850–58-specific T cell clone ZH-104/7. We confirmed by Western blot analysis that LMP-7, MECL-1, and LMP-2 were detected similarly in NW-Mel-16 cells upon IFN-γ treatment and in EBV-transformed B-LCL cells (Fig. 4a). It was shown previously that the latter express the immunoproteasome constitutively (27). As depicted in Fig. 4b, clone ZH-104/7 efficiently recognized tumor cells both treated or not with IFN-γ, suggesting that processing of RAB3850–58 peptide is not affected by the replacement of the standard proteasome. The IFN-γ treatment did not affect the level of RAB38 expression, as measured by real-time PCR and Western blot analysis (data not shown). Thus, in contrast with other tumor-specific antigenic peptides, RAB3850–58 peptide is efficiently processed by the two proteasome types. Additionally, to document that the proteasome is indeed responsible for the generation of RAB3850–58 peptide, EBV-transformed B-LCL cells were electroporated with mRNA encoding for full-length RAB38 and treated with lactycystin, which affects Ag processing by the standard proteasome. The IFN-γ secretion of RAB3850–58-specific T cell clone ZH-104/7 was reduced by 50%, further indicating that its processing.
cells in patient MeM297 collected in October 2001 (before IL-2) corresponded to \( \sim 2 \times 10^{-1} \) circulating CD8 T cells (0.02%), which is clearly above background values (<0.01%). Interestingly, the frequency of specific T cells increased threefold (\( \sim 6 \times 10^{-4} \)) while the patient was treated with IL-2 until January 2003 (Fig. 5). The vast majority of those RAB38so,ss-specific CD8 T cells displayed an Ag experienced CCR7 CD45RA+ phenotype, corresponding to the recently described effector memory T cell subset (30). In addition, 10 melanoma patients were evaluated for the response to RAB38so,ss by short-term culture of PBMCs in the presence of exogenously added peptide RAB38so,ss and cytokines. Specific CD8 T cells could readily be identified in 2 of 10 patients (data not shown). Taken together, our data indicate that RAB38so,ss-specific CD8 T cells have been expanded previously in vivo in the response to the autologous tumor.

### Discussion

RAB proteins form the largest family of the Ras superfamily of small GTP-binding proteins involved in vesicular trafficking pathways (31). The melanocyte differentiation Ag RAB38 identified previously by the SEREX approach (16) is characterized mainly by 1) a highly restricted expression in normal melanocytes and melanoma lesions, and 2) strong immunogenicity reflected by spontaneous humoral immune responses in a large proportion of melanoma patients (17). In this study, we demonstrate for the first time that RAB38 can elicit a spontaneous CD8 T cell response in addition to Abs. To date, only a few tumor Ags, e.g., NY-ESO-1, have been reported that may elicit immune responses involving both humoral and cellular effectors (32, 33).

The short protein sequence of RAB38 made it possible to combine in vitro sensitization using overlapping peptides with in silico prediction of potential HLA-A2 ligands, coined “reverse immunology” (19). The peptides that were predicted to bind with high affinity based on appropriately positioned anchor residues were tested further for their immunogenicity. RAB38so,ss-specific CD8 T cell responses were readily detectable in the PBMCs of three of four melanoma patients, who have been short-term stimulated with RAB38so,ss peptide. Interestingly, only one of those patients exhibited an anti-RAB38 humoral response, which would implicate that CD8 T cell responses may take place in the absence of specific humoral responses. This is in line with observations with respect to cellular and humoral NY-ESO-1 responses (34). However, only a larger series of seropositive and seronegative patients will give a more comprehensive picture of this issue. Although reverse immunology has successfully led to the identification of numerous epitopes, it revealed some peptides, however, that are unlikely to be naturally processed as demonstrated by the poor recognition of target cells endogenously expressing the Ag (20, 35, 36). Thus, we chose to perform in vitro digestion assays of a synthetic long oligopeptide encompassing RAB38so,ss peptide, and recognition experiments with RAB38so,ss-specific T cells using COS-7 and HEK293 cells transfected with the RAB38 sequence and, more importantly, melanoma cells endogenously expressing RAB38 as targets. These assay confirmed the adequate generation of RAB38so,ss peptide by the cellular processing machinery. As the N-terminal extended decapeptide RAB38so,ss, however, is recognized with the same functional avidity, either of the two peptides may be presented by tumor cells. It should be emphasized that recognition by CD8 T cell clones was specifically directed toward RAB38. Undesirable cross-recognition against cells expressing ubiquitously expressed RAB proteins that share >75% amino acid sequence identity, e.g., RAB32, was ruled out by TAP-deficient T2 cells pulsed with a RAB32 peptide exhibiting the highest sequence homology to RAB38so,ss.
Representing self-proteins, intentional targeting of melanocyte differentiation Ags, e.g., RAB38, may result in inducing autoimmunity. This is further underlined by our finding that even tumor cells with expression levels lower or comparable to melanocytes are efficiently recognized by RAB38-specific CD8 T cells. However, it needs to be emphasized that although abundantly expressed in melanoma lesions, RAB38 expression is limited strictly to melanocytes (17). As demonstrated for one patient who received infusion of CD8 T cell clones specific for another melanocyte differentiation Ag, Melan-A/MART-1, postinfusion biopsies of the skin showed clinical signs of vitiligo and localization of the infused Melan-A/MART-1-specific CD8 T cell clones (37). Given the autoimmune toxicity and temporary clinical response in this patient, tumor immunity against self-Ags can be regarded as a special case of autoimmune reaction against transformed melanocytes. In this regard, effective anti-tumor immunity induced by RAB38 peptide based vaccinations may be limited to variable extents by the process of central and peripheral tolerance that are essential to avoid the potentially devastating effects of autoimmunity.

To assess spontaneously occurring CD8 T cell responses to RAB38 we used fluorescent multimers to stain circulating lymphocytes from HLA-A2 melanoma patients. Although the frequencies of RAB38-specific CD8 T cells were generally below the multimer detection limit, one patient (MeM297) exhibited a directly ex vivo detectable frequency. As recently demonstrated for the vast majority of Ag experienced CD8 T cells specific for Melan-A/MART-1 (10), RAB38-specific CD8 T cells were CD45RAlow CCR7+, a phenotype proposed to characterize effector memory T cells potentially able to home into inflamed tissue (30). Importantly, this differentiation stage indicates encounter and activation with naturally processed RAB3850–58 peptide presented on APCs in vivo in this patient. Remarkably, the high frequency of memory effector RAB38-specific CD8 T cells even increased under low-dose IL-2 treatment. Why those cells failed to effectively prevent tumor progression in this patient is difficult to evaluate, as various mechanisms underlying the cancer immunoeediting process could intervene at that stage. Encouraged by the growing appreciation that tumors are capable to create an immunosuppressive situ network that shields tumor cells from T cell immune attack (38), it is of our future interest to further dissect characteristics of RAB38-specific immune responses, e.g., T cell function, present at tumor sites. Importantly, in sharp contrast to the large repertoire of Melan-A-specific CD8 T cells (18), RAB38-specific CD8 T cells are not identifiable ex vivo in healthy donors. However, these cells become detectable in some healthy individuals, but only after repetitive in vitro stimulations. Similar findings have been documented previously for other epitopes, e.g., SSX-2 (39). Additional experiments will aim at assessing functional avidity of RAB38-specific CD8 T cells in those tumor-free individuals.

The proteasome is a multicatalytic protease complex that plays a critical role in the Ag processing pathway. In contrast to the standard proteasome expressed in all somatic cells, lymphoid cells (including myeloid-derived DCs) or cells under the influence of IFN-γ express the immunoproteasome that exhibits an altered cleavage site preferences (40). In contrast to other approaches based on in vitro proteasomal digestion (41), we initially used here the standard proteasome to confirm proper processing of RAB3850–58 peptide. For some tumor Ags, however, immunoproteasomes negatively affect epitope processing (25, 42). In contrast, other antigenic peptides appear to be produced more efficiently by the immunoproteasome (26). Thus, those peptides that are poorly processed by immunoproteasomes may not be generated at all or only poorly generated by professional APCs. We show in this study that RAB3850–58 peptide is processed by both proteasome types. Importantly, this implies that, even in an IFN-γ rich environment, e.g., present at tumor sites heavily infiltrated with T cells, RAB38 can be presented on melanoma cells. In addition, given the high expression of immunoproteasomes in mature DCs, RAB3850–58 peptide may also be involved in initiating the immune response to RAB38. However, as a highly diverse set of genes representing tissue restricted self Ags are promiscuously expressed in thymic cells (43), it is possible that weak expression of RAB38 in the thymus could play a role in specific thymocyte selection processes.

Overall, in this study, we have described the first characterization of a natural CD8 T cell response against the melanocyte differentiation Ag RAB38. Aside from important implications for clinical trials of vaccination with RAB38 immunogenic molecules, the data presented here confirm and extend our previous findings supporting the immunogenicity of RAB38 in melanoma patients. It thus appears that RAB38, as shown so far only for some Ags, can elicit immune responses involving both humoral and cellular effectors. The role of CD4 T cells in generating and maintaining anti-RAB38 immune responses is currently investigated in our laboratory.

Acknowledgments

We thank Dr. I. Luescher (Ludwig Institute for Cancer Research, Epalin- ges, Switzerland) for synthesis of multimers and Dr. K. Osanai (Depart- ment of Internal Medicine, Kanazawa Medical University, Ishikawa, Japan) for providing anti-RAB38 Ab. We thank Conny Schneider, Heidi Mattlin, Renate Wenig, Julia Karbach, Claudia Frei, Nicole Lévy, and Anne-Lise Peitrequin for their excellent technical assistance.

Disclosures

The authors have no financial conflict of interest.

References

RAB38-SPECIFIC CD8 T CELLS IN MELANOMA PATIENTS


