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LETTER TO THE EDITOR

Cancer-testis antigen MAGE-C2/CT10 induces spontaneous CD4$^+$ and CD8$^+$ T-cell responses in multiple myeloma patients

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Multiple myeloma (MM) is the second most common hematologic malignancy with an incidence of 15 000 new cases per year in the United States alone, and is characterized by the proliferation of a malignant plasma cell clone within the human bone marrow (BM). The expansion of neoplastic plasma cells producing a monoclonal immunoglobulin typically results in BM failure with anemia, skeletal involvement with lytic bone lesions and consecutive hypercalcemia. The excessive production of paraprotein can lead to renal failure and recurrent bacterial infections due to a decrease in polyclonal immunoglobulins.[1] Recently, new treatment strategies including immunomodulatory thalidomide and its derivatives and the proteasome inhibitors bortezomib and carfilzomib have been developed, and significantly improve the outcome of MM patients.[2,3] However, despite these advances, myeloma remains an incurable malignancy with a median overall survival of only 5 years and, accordingly, additional treatment options are urgently warranted.

One way to eradicate even chemotherapy-resistant disease could be the engagement of immune effector cells targeting antigens that are specifically expressed by the malignant plasma cells. MM is probably the cancer with the most frequent expression of cancer-testis antigens (CTA), a class of tumor antigens characterized by its tumor-restricted expression and high immunogenicity. MAGE-C2/CT10[4,5] is among the CTA that are most frequently expressed in MM, being detectable in the BM of the majority of myeloma patients,[6–8] and it seems very likely that, as in other tumor types,[9] MAGE-C2/CT10 also promotes the

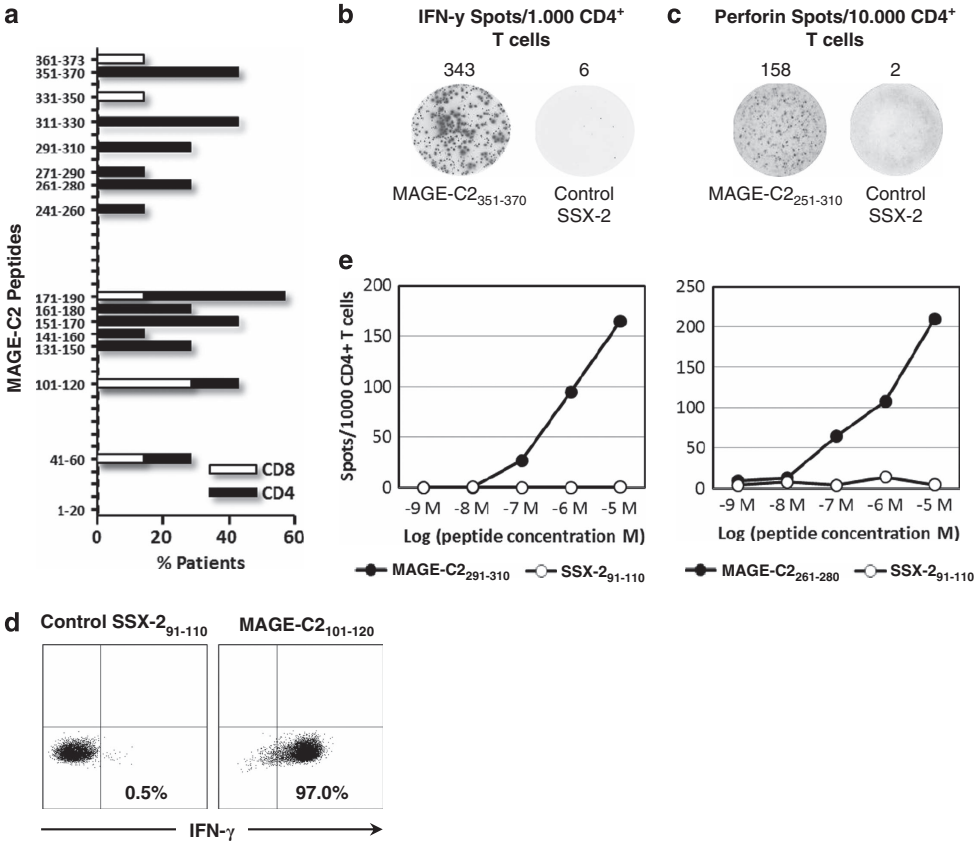

Figure 1. Characterization of T-cell responses against MAGE-C2/CT10. (**a**) Percentages of seven patients showing T-cell responses (black bars = CD4$^+$, white bars = CD8$^+$) against a given peptide epitopes of MAGE-C2/CT10 as assessed by IFN-γ ELISPOT. (**b**) Exemplary IFN-γ ELISPOT of a MAGE-C2/CT10-specific T-cell line after exposure to the cognate peptide MAGE-C2$_{351-370}$ (left) or control peptide SSX-2$_{91-110}$ (right). (**c**) Perforin ELISPOT of bulk T cells from patient UKE-1207 after exposure to the cognate MAGE-C2/CT10 antigen (left, pool of peptides MAGE-C2$_{251-270}$ to MAGE-C2$_{291-310}$) or control peptide SSX-2$_{91-110}$. (**e**) Peptide titration experiment for the assessment of antigen affinity of two MAGE-C2/CT10-specific CD4$^+$ T-cell clones. The mean number of IFN-γ spots after exposure to the cognate (black dots) or control peptide (open dots) is indicated. (**d**) Exemplary FACS dot blot of a MAGE-C2/CT10-specific CD4$^+$ T-cell clone after exposure to the cognate peptide MAGE-C2$_{101-120}$ (left) or control peptide SSX-2$_{91-110}$ (right). Intracellular IFN-γ production is shown on the *x*-axis.

Table 1. Identification of TCRs for the most relevant MAGE-C2/CT10-specific T-cell clones

TCC no.	*TRBV*	*TRBJ*	*TRBD*	*CDR3 beta*	*TRAV*	*TRAJ*	*CDR3 alpha*	*MAGE-C2 peptide specificity*
3	7-3*01	2-3*01	2*02	CASSLTGSAPTDTQYF	8-1*01	37*01	CAVKVNNAGNMLTF	311–330
10	6-1*01	2-4*01	1*01	CASSEHGQGVPAKNIQYF	8-1*01	43*01	CAVNARNNNDMRF	171–190
20	7-8*01	1-6*01	1*01	CASSLDLDRGNSPLHF	2*01	29*01	CAVEAYSGNTPLVF	171–190
24	2*01	2-7*01	1*01	CASSLGGQLREQYF	26-1*01	47*01	CIVRGLREYGNKLVF	291–310
33	5-1*01	1-2*01	1*01	CASSLALKAEDGYTF	3*01	9*01	CAVRDPHTGGFKTIF	241–260
35	7-7*01	2-7*01	2*01	CASSQNKVGEQYF	12-2*02	8*01	CAVNEDFQKLVF	261–280

Abbreviations: CDR3, complementarity determining region 3; TCC, T-cell clone; TRAV/TRBV, T-cell receptor alpha/beta chain. TCR alpha and beta chain compositions according to the IMGT nomenclature are given.

malignant phenotype in myeloma. However, it has remained unclear whether spontaneous immune responses against autologous tumor cells expressing this target occur in myeloma patients. This is an unfortunate situation because once effective MAGE-C2/CT10-specific T cells have been identified in patients with MM, the respective T-cell receptors (TCRs) could be isolated and transduced into primary T cells of patients who lack a natural immune response capable of controlling the malignancy—an approach that has successfully been applied to patients with solid tumors.[10] Here, we have for the first time performed an analysis of $CD4^+$ and $CD8^+$ T-cell responses against MAGE-C2/CT10 in myeloma patients. One major goal of our analysis was to isolate specific high-quality TCRs from myeloma patients in order to make them available for future therapeutic options incorporating the adoptive transfer of genetically modified T cells.

After they had provided written informed consent, blood samples were collected according to the Declaration of Helsinki from seven myeloma patients who evidenced MAGE-C2/CT10 expression in their BM. We then prepared peripheral blood mononuclear cells by density centrifugation and separated $CD4^+$, $CD8^+$ and $CD4^-/CD8^-$ fractions using magnetic beads. $CD4^+$ and $CD8^+$ T cells, respectively, underwent a single round of MAGE-C2/CT0-specific stimulation for 10–20 days using $CD4^-/CD8^-$ antigen-presenting cells pulsed with pools of overlapping MAGE-C2/CT10 20 mer peptides (10 aa overlap) spanning the entire sequence of the antigen. Methods and Materials used in this study are the same as the ones applied in our previous studies.[8]

Remarkably, in an interferon-γ ELISPOT read-out assay, we detected MAGE-C2/CT10-specific $CD4^+$ memory T-cell responses, and sometimes also $CD8^+$ T-cell responses, in all patients tested (Figures 1a and b). There seemed to be an immunodominant region within the amino acid sequence 131–190 of the whole MAGE-C2/CT10 protein. All patients evidenced $CD4^+$ and/or $CD8^+$ T-cell responses against one or more peptides within this region and T cells reacting against two distinct peptide epitopes, MAGE-C2/$CT10_{171–190}$ and MAGE-C2/$CT10_{151–170}$, were detectable in four and three out of seven patients, respectively. Other peptide epitopes frequently recognized were MAGE-C2/$CT10_{101–120}$, MAGE-C2/$CT10_{311–330}$ and MAGE-C2/$CT10_{351–370}$ each having evoked memory T-cell responses in three out of seven patients (Figure 1a).

From T-cell lines specific for the most prominent MAGE-C2/CT10 epitopes, we next generated a total of 10 different T-cell clones. All T-cell clones were able to release IFN-γ upon exposure to their cognate antigen (Figures 1b and d). Intriguingly, $CD4^+$ T-cell clones also reacted with perforin secretion upon encounter with the cognate antigen (Figure 1c), suggesting cytolytic potential even of the T-helper clones isolated. In addition to the expression of a memory effector T-cell phenotype, a given T cell's antigen affinity is a critical factor for their suitability for adoptive immunotherapy. Importantly, we could demonstrate that all our MAGE-C2/CT10-specific T-cell clones had affinities in the nanomolar range (Figure 1e) making their TCRs promising candidates for the transduction into primary T cells of cancer patients. Based on these findings, we finally confirmed clonality for six T-cell specificities by sequencing the respective TCR chains (Table 1), making these highly promising candidates available for future immunotherapeutic approaches in myeloma and maybe also in other malignancies.

It has been shown for patients with MM that lymphocytes infiltrating their BM have the potential to target myeloma cells and their precursors,[11] T cells recognizing autologous tumor have repeatedly been isolated from patients with MM[12–14] and in the context of an allogeneic stem cell transplantation a T-cell-mediated graft-versus-myeloma effect becomes clinically apparent.[15] Overall, the adaptive immune system is clearly capable of recognizing and attacking malignant plasma cells in patients with MM, however, it has remained unclear which antigens exactly are recognized by such tumor-targeting T cells. Here, we have shown for the first time that a large proportion of myeloma patients evidence T cells specific for the myeloma-restricted antigen MAGE-C2/CT10. We have also demonstrated that the respective T cells are fully functional and display a high affinity for their target antigen. Finally, we have described in detail the TCRs used by the MAGE-C2/CT10-specific T-cell clones isolated from myeloma patients with MAGE-C2/CT10-positive disease. Hopefully, these combined findings will contribute to the development of myeloma-targeting immunotherapies—preferably the adoptive transfer of T cells transduced with anti-MAGE-C2/CT10 TCRs.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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