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SHORT REPORT

Functionally Different HA-1 Specific T Cells Use the Same TRBV7-9: A Snake in the Grass for T Cell Receptor Transfer Studies

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Abstract: New developments in cellular adoptive immunotherapy have led to tailor made therapies for cancer patients. One of the latter therapies is tumor antigen–specific T cell receptor (TCR) transfer enabling recognition of, for example, the hematopoietic restricted minor histocompatibility (H) antigen HA-1. Since HA-1 is also expressed on leukemic and leukemic progenitor cells, transfer of in vitro–generated HA-1 CTL or usage of HA-1 TCR-modified T cells will contribute to an anti-leukemic response. The fact that all thus far analyzed HA-1–specific T cells express the same TCR Vbeta TRBV7-9, makes this TCR an attractive candidate for TCR transfer studies. Here we show however different cytolytic capacity among the TRBV7-9 expressing HA-1–specific T cells. Consequently, functional in vitro studies of the relevant T cells prior to TCR transfer are highly recommended.

Keywords: minor histocompatibility antigen, graft versus leukemia, HA-1, T cell receptor, cytotoxic T cell

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Introduction

Graft versus leukemia (GVL) is the curative response of hematopoietic stem cell transplantation (HSCT) for hematological malignancies. One of the targets in the GVL response is the hematopoietic-restricted minor H antigen HA-1.[1] Minor H antigens are polymorphic peptides presented in the context of HLA class I or HLA class II molecules. In HLA-matched HSCT, minor H antigen disparities between donor and patient can result in detrimental graft versus host disease (GVHD) but more importantly are crucial in the GVL response. Minor H antigens can be either broadly expressed or are hematopoietic system–restricted.[2] HA-1 is one of the most well-known and well-characterized hematopoietic system–restricted minor H antigens; it is expressed on normal hematopoietic cells, leukemic cells, and on a majority of solid tumor cells.[3] HA-1^{H}–specific T cells can be observed after HSCT in HA-1^{H} (the immunogenic phenotype) patients transplanted with HA-1^{RR} (the non-immunogenic phenotype) donors.[4] Associations between HA-1 mismatch and GVL and better transplantation outcome[5] suggest HA-1^{H} as a promising target in HSCT-based immunotherapeutical studies, such as vaccination[6] or adoptive immunotherapy with HA-1^{H}–specific cytotoxic T cells (CTL) or with HA-1^{H}–specific TCR modified T cells.[7,8]

An interesting feature of HA-1^{H} CTLs is their restricted TCR usage. Notably, HA-1^{H} CTL isolated from in vivo immunized individuals as well as HA-1^{H} CTLs generated in vitro use the same TRBV7-9 (according to the current nomenclature http://www.imgt.org)[9,10] for recognizing the HLA-A2/HA-1 target. More recently, we isolated both HA-1^{H}–specific CTLs and HA-1^{H}–specific regulatory T cells (Treg) from patients and from healthy subjects.[11,12] In the latter studies, low HA-1^{H}–specific tetramer (tetramer^{low}) staining intensity was associated with regulatory T cell activity. Importantly, in vitro, these tetramer^{low} staining T cells, presumable of low avidity, only lysed peptide pulsed target cells, whereas the tetramer^{high} staining T cells, presumable of high avidity, were highly cytotoxic and lysed both natural ligand and peptide-loaded target cells in vitro. Along with the identification of HA-1^{H}–specific Treg, we questioned whether the latter T cells might have the same restricted Vβ usage as the functionally different CTL for recognizing the same ligand.[9,10] Notably, in our previous study, TRBV7-9 usage was observed in one in vitro generated low avidity HA-1^{H}–specific T cell clone with tetramer^{low} staining and recognition of HA-1^{H} peptide–pulsed target cells only.[10] If indeed HA-1^{H}–specific Treg have the same Vβ usage as the HA-1^{H}–specific CTLs, it has important clinical consequences. Namely, HA-1^{H} tetramer staining and TRBV7-9 expression are the landmark of potent HA-1^{H} CTLs used for HA-1 TCR transfer studies[8] and for ex vivo generation of HA-1^{H} T cells for adoptive T cell therapy after stem cell HSCT.[13] The goal of this study was to extend our initial and single observation that the same TCR Vβ is used by functionally different HA-1^{H} specific T cells.[10]

Methods

Isolation and culturing of HA-1–specific T cells

HA-1^{H} tetramer staining CD8^{pos} T cells[14] were isolated from 6 HA-1^{RR} healthy donors and from a patient after HLA-identical HA-1 mismatched HSCT. Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Isopaque density gradient centrifugation and depleted for various cell subsets using CD4, CD14, CD16 and CD19 MACS beads according to manufacturer's instructions (Miltenyi Biotec, Bergisch Gladbach, Germany). The depleted fraction was subsequently stained with validated[14] phycoerythrin (PE)-conjugated HLA-A2/HA1^{H} and allophycocyanin (APC)-conjugated CD8 (BD Biosciences, Breda, The Netherlands). CD8^{pos} tetramer^{pos} T cells were isolated by a FACS-ARIA cell sorter (Becton, Dickinson and company, Breda, The Netherlands) and collected single cell per well in 96-well plates containing irradiated HA-1^{RR} feeder cells in Iscove's Modified Dulbecco's Medium (IMDM, Lonza, Verviers, Belgium) with 10% pooled human serum (HS), 1% leucoagglutinin (Leuco-A, Sigma-Aldrich, Zwijndrecht, The Netherlands) and 25 U/mL recombinant interleukin-2 (IL-2, Cetus, Emeryville, CA, USA). After initial stimulation, expanding T cell clones were harvested. Tetramer^{pos} T cell clones were further expanded according to the stimulation protocol described above.

Functional assays

T cell clones were kept in IMDM 10% HS 25 U/mL IL-2 overnight before testing of cytotoxicity in a

standard chromium release assay or 3HThymidine proliferation assay.[15] In brief, for cytotoxicity 2500 $^{51}Cr^{-}$ labeled target cells (HLA-A2/HA-1^{H} Epstein Barr virus transformed lymphoblastoid cell lines (EBV-LCLs), HLA-A2/HA-1^{RR} EBV-LCL or HLA-A2/HA-1^{RR} EBV-LCL loaded with HA-1^{H} peptide) were incubated with serial dilutions of T cells. Supernatants were harvested after 4 hours of incubation for gamma counting. Percentage-specific lysis was calculated as follows: (experimental release − spontaneous release)/(maximal release − spontaneous release) × 100%. In the proliferation assay, 50,000 irradiated EBV-LCL were co-cultured with 10,000 T cells for 48 hours. Thereafter, 3H-thymidine was added and cells were kept overnight before harvesting and counting 3H-thymidine incorporation.

All T cell clones were been tested in two independent assays.

T cell receptor analyses

From all functional HA-1^{H} tetramer^{pos} T cells, RNA was isolated using TRIzol (Life Technologies, Bleiswijk, The Netherlands). One µg of RNA was used to synthesize cDNA using oligodT-primers (Life Technologies). cDNA was amplified by a 35 cycle PCR using 32 TRBV family–specific 5′-primers as descriped before.[9,10] Dominant PRC fragments were subsequently sequenced (Baseclear, Leiden, the Netherlands) to identify the specific Vβ, NDN, and J region and to determine clonality.

Results

Tetramer staining profiles, functional data, and the TCR Vβ usage of 15 T cell clones of 5 individuals from whom functional T cell clones were isolated are summarized in Table 1. Both the number of obtained HA-1^{H}–specific T cell clones and the tetramer staining intensity differed among the individuals (Table 1, Fig. 1A). The 15 T cell clones could be divided into two functionally different types of HA-1^{H} tetramer^{pos} T cell clones, that is, the type 1 cytotoxic T cell (CTL) clone that lysed both HA-1^{H} natural ligand-expressing target cells (n = 5) and HA-1^{RR} target cells exogenously loaded with the HA-1^{H} peptide. The type 2 CTL clones that lysed HA-1^{RR} target cells exogenously loaded with the HA-1^{H} peptide only (n = 10).

All 15 T cell clones were also analyzed for their proliferative capacities. Although CD8^{pos} T cells are not highly proliferative, distinct reaction patterns comparable to the cytolytic patterns were clearly observed. Namely, the 5 type 1 T cell clones proliferated on the natural ligand and on the peptide-loaded ligand; the 10 type 2 T cell clones proliferated solely when stimulated with the peptide-loaded ligand (data not shown).

In line with our earlier observations,[9,10] all 5 type 1 CTL clones recognizing the HA-1^{H} natural ligand-expressing target cells used the restricted TCR Vβ chain TRBV7-9 (Table 1). Eight out of 10 type 2 CTL clones, recognizing only peptide-pulsed target

Table 1. Overview of functional HA-1^{H}–specific T cell clones.

Donor ID	Clone ID	HA-1^{H} tetramer staining intensity¥	lysis* of male natural ligand	lysis* of peptide loaded cells	TRBV	Vbeta	NDN	TRBJ
1	R1-51	Normal	–	+	7-9	AMYLCASS	TGLA	1-1
2	PW1-108	Low	–	+	7-9	AMYLCASS	LLAGGLV	2-1
	PK1-107	Normal	+	+	7-9	AMYLCASS	LVVGD	2-7
	PK1-141	Normal	–	+	7-9	AMYLCASS	LGAAY	2-7
3	FH1-50	Low	+	+	7-9	AMYLCASS	TVTGVD	1-2
	FH3-79	Low	–	+/–	7-9	AMYLCASS	FVSL	2-1
4	1W10	Low	–	+	7-3	AVYLCASS	QRQGRR	2-1
	3W85	Normal	–	+	7-9	AMYLCASS	QRAGG	2-5
	1W33	Normal	–	+	7-9	AMYLCASS	LVGR	1-4
	3W13	Normal	–	+	7-9	AMYLCASS	SHAGG	1-4
5^	C2	Normal	+	+	7-9	AMYLCASS	LISG	1-4
	A1	High	+	+	7-9	AMYLCASS	LVQ	1-3
	B12	High	+	+	7-9	AMYLCASS	IKVQG	1-1
	D11	High	–	+	7-9	AMYLCASS	LTLL	2-3
	A9	High	–	+	3-1	AVYFCASS	QKGP	2-1

Notes: ¥According to the examples given in Figure 1A; *specific lysis, + = ≥ 25%, – = < 25%; ^patient after HSCT.

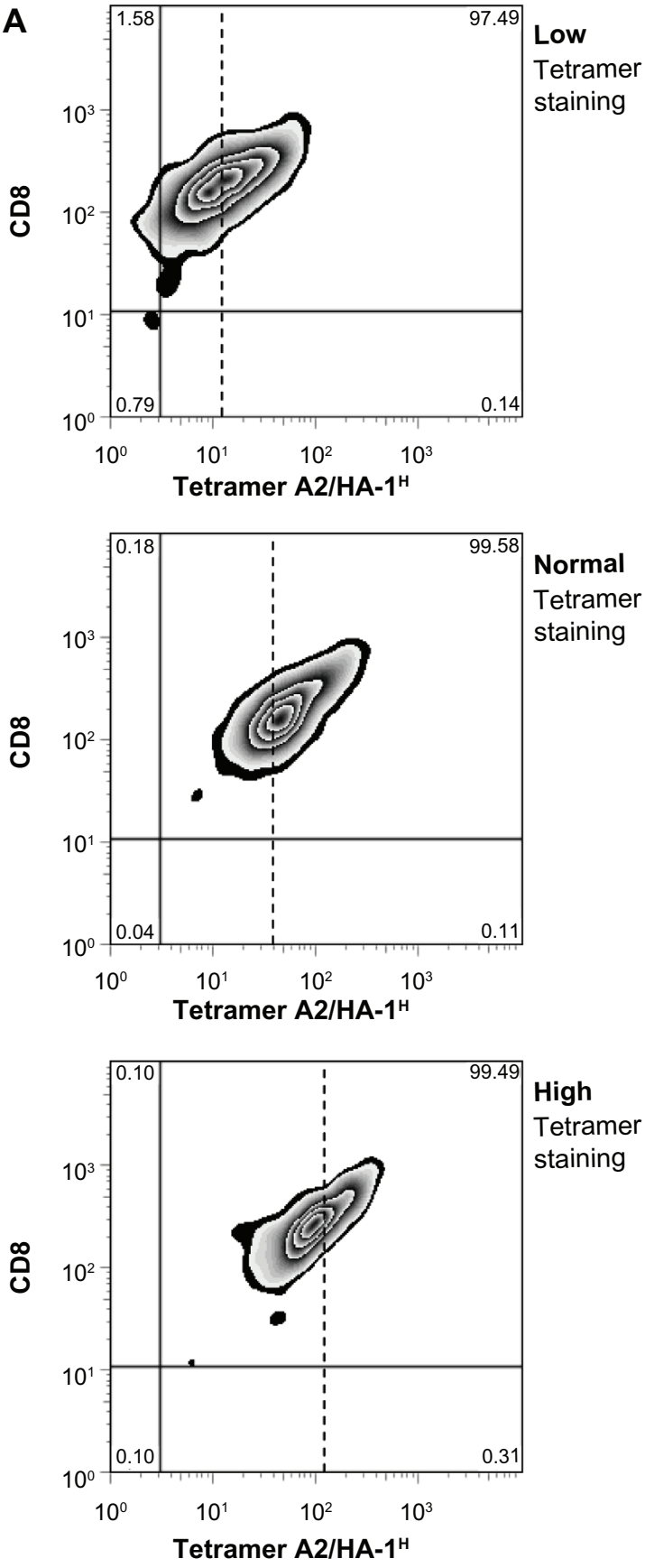

Figure 1. (*Continued*)

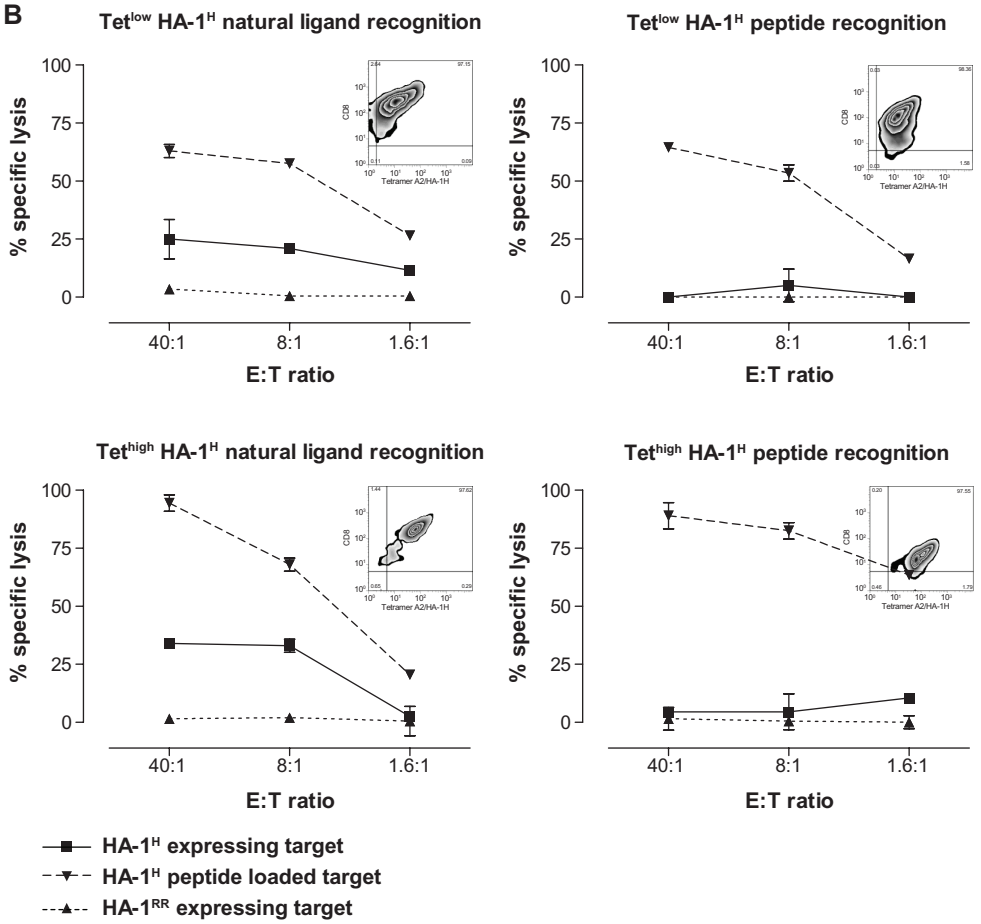

Figure 1. (**A**) After isolation of tetramer^{pos} T cells, three levels of tetramer staining intensity of T cell clones could be identified, that is, low, normal and high tetramer staining. (**B**) All T cell clones which remained tetramer positive after several rounds of expansion were tested for cytotoxic function.
Notes: The antigen-specific response was measured in a chromium release assay. Herein, the clones were tested against EBV-LCL naturally expressing HA-1^{H} or HA-1^{RR} EBV-LCL loaded with the HA-1^{H} peptide. The upper panel shows representative examples of single experiments of tetramer low staining (Tet^{low}) T cell clones, which recognize the natural ligand and HA-1^{H} peptide-loaded target cells or only recognize HA-1^{H} peptide-loaded target cells. The lower panel shows examples of similar functional T cell clones, which all have a high tetramer staining (Tet^{high}) intensity. The data are shown as the mean of duplicate samples.

cells, used the TRBV7-9 as well. From 3 out of 5 donors we were able to isolate both the type 1 and the type 2 HA-1^{H}–specific T cell clones as is illustrated by their differential NDN and joining region expression.

Conclusion and Discussion

In summary, HA-1^{H} tetramer^{pos} T cell clones that lyse HA-1^{H} natural ligand-expressing target cells in vitro, the type 1 CTL, all share the restricted TCR Vβ TRBV7-9. It is expected that these high avidity CTL clones are most potent in vivo and thus relevant for the graft versus leukemia reactivity.[16,17]

Yet, most (8 out of 10) of the type 2 CTL that do not lyse the natural ligand but do specifically lyse the exogenous HA-1^{H} peptide-loaded target cells also use the restricted TCR Vβ TRBV7-9. What the in vivo function of these latter T cells is remains speculative. Notably, in one of our former clinically related studies, CD8^{pos} tetramer^{low} peptide–specific T cell lines demonstrated a regulatory phenotype and suppressive capacity.[11,12] In this underlying study, the type 2 HA-1^{H} T cell clones showed tetramer^{high} staining, HA-1^{H} peptide–specific cytolytic capacity but did not express any of the CD8 Treg-associated markers like CTLA-4, TGFβ, or GITR (data not shown). Thus, tetramer staining intensity is not the sole marker for antigen avidity. Whether HA-1^{H} peptide–specific T cells, expressing TRBV7-9, have regulatory functions in vivo remains to be seen.

Since it is unclear what the in vivo function is of peptide-specific T cells, thorough in vitro functional

analyses of the relevant T cell clone should precede the use of TRBV7-9 for TCR transfer for adoptive T cell therapy. As long as there exist no phenotypic makers to determine a Treg phenotype, the usage of a particular HA-1^{H} T cell clone for TRBV7-9 transfer or for cellular adoptive immunotherapy should among others be based on their strong natural ligand-specific lysis in vitro.

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Competing Interests
Author(s) disclose no potential conflicts of interest.

Author Contributions
Conceived and designed the experiments: MD, ES, JP, EB, EG. Analyzed the data: MD, JP, EG. Wrote the first draft of the manuscript: MD, EG. Contributed to the writing of the manuscript: WB. Agree with manuscript results and conclusions: MD, ES, JP, EB, WB, EG. Jointly developed the structure and arguments for the paper: MD, WB, EG. Made critical revisions and approved final version: MD, WB, EG. All authors reviewed and approved of the final manuscript.

Disclosures and Ethics
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