CHAPTER VI

Functionally different HA-1 specific T cells use the same TRBV7-9; a snake in the grass for T cell receptor transfer studies

Miranda P. Dierselhuis, Ellen Schrama, Jos Pool, Els C. Blokland, William J. Burlingham and Els Goulmy

Immunology and Immunogenetics Insights 2012;4:1–7

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 is highly recommended.

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 cells3. 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*, all 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 Vbeta 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 Vbeta 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 SCT[13].

The goal of this study was to extend our initial and single observation that the same TCR Vbeta 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 validated14 Phycoerythrin (PE)-conjugated HLA-A2/HA1H and allophycocyanin (APC)-conjugated CD8 (BD Biosciences, Amsterdam, the Netherlands). CD8^{pos}tetramer^{pos} T cells were isolated by a FACS-ARIA cell sorter (Becton Dickinson) and collected single cell per well in 96-well plates (Greiner Bio-One, the Netherlands) 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, 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 51Cr 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 hour of incubation for gamma counting. Percentage specific lysis was calculated as follows: (experimental release - spontaneous release)/(maximal release – spontaneous release) x 100%. In the proliferation assay 50,000 irradiated EBV-LCL were co-cultured with 10,000 T cells for 48 hours. Thereafter ^{3}HThymidine was added and cells were kept overnight before harvesting and counting ^{3}HThymidine incorporation. All T cell clones have been tested in two independent assays.

T cell receptor analyses

From all functional HA-1H tetramerpos T cells RNA was isolated using TRIzol (Life Technologies, Bleiswijk, The Netherlands). 1 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 frag-

ments were subsequently sequenced (Baseclear, Leiden, The Netherlands) to identify the specific Vbeta, NDN and J region and to determine clonality.

Results

Tetramer staining profiles, functional data and the TCR VBeta 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, figure 1A). The 15 T cell clones could be divided into two functionally different types of HA-1^{H} $tetramer^{pos}$ T cell clones; i.e.: 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

Table 1. Overview of functional HA-1H specific T cell clones

Donor ID	Clone ID	HA-1H 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

¥ According to the examples given in figure 1A
* specific lysis, + = ≥ 25%, - = < 25%
^ patient after HSCT

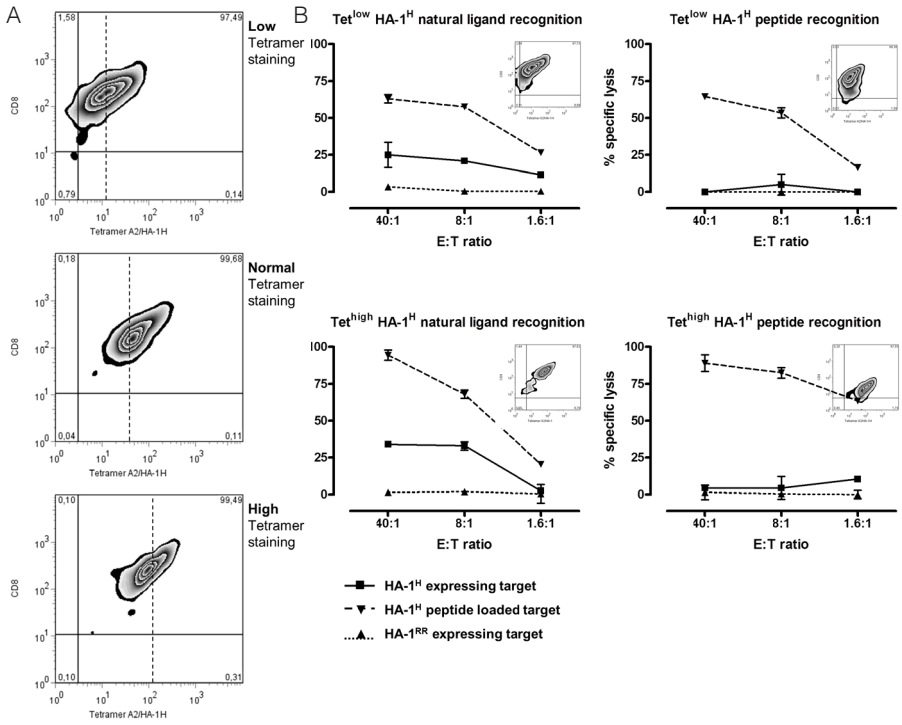

Figure 1.
A) After isolation of tetramerpos T cells, three levels of tetramer staining intensity of T cell clones could be identified, i.e. low, normal and high tetramer staining.
B) All T cell clones which remained tetramer positive after several rounds of expansion were tested for cytotoxic function. The antigen specific response was measured in a chromium release assay. Herein the clones were tested against EBV-LCL naturally expressing HA-1H 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 (Tetlow) T cell clones, which recognize the natural ligand and HA-1H 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 (Tethigh) intensity. The data are shown as the mean of duplicate samples.

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 Vbeta chain TRBV7-9 (table 1). Eight out of 10 type 2 CTL clones, recognizing only peptide pulsed target 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-1H natural ligand expressing target cells in vitro, the type 1 CTL, all share the restricted TCR Vbeta 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, also most (8 out of 10) of the type 2 CTL that do not lyse the natural ligand but do specifically lyse the exogenous HA-1H peptide loaded target cells, do use the restricted TCR Vbeta TRBV7-9 as well. What the in vivo function of these latter T cells is remains speculation. Notably, in one of our former clinical 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, TGFbeta 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 amongst others be based on their strong natural ligand specific lysis *in vitro*.

Acknowledgements

The authors thank the HLA tissue-typing laboratory of the Department of Immunohematology and Blood Transfusion for performing HLA and minor H antigen genotyping; M.A.W.G. van der Hoorn, G.M. de Roo and P.P.G. van der Holst for excellent technical assistance in the FACS sorting experiments.

Reference List

1. den Haan JM, Meadows LM, Wang W et al. The minor histocompatibility antigen HA-1: a diallelic gene with a single amino acid polymorphism. Science 1998;279:1054-1057.
2. de Bueger MM, Bakker A, van Rood JJ, Van der Woude F, Goulmy E. Tissue distribution of human minor histocompatibility antigens. Ubiquitous versus restricted tissue distribution indicates heterogeneity among human cytotoxic T lymphocyte-defined non-MHC antigens. J Immunol 1992;149:1788-1794.
3. Hambach L, Goulmy E. Immunotherapy of cancer through targeting of minor histocompatibility antigens. Curr Opin Immunol 2005;17:202-210.
4. Marijt WA, Heemskerk MH, Kloosterboer FM et al. Hematopoiesis-restricted minor histocompatibility antigens HA-1- or HA-2-specific T cells can induce complete remissions of relapsed leukemia. Proc Natl Acad Sci U S A 2003;100:2742-2747.
5. Mutis T, Brand R, Gallardo D, van BA, Niederwieser D, Goulmy E. Graft-versus-host driven graft-versus-leukemia effect of minor histocompatibility antigen HA-1 in chronic myeloid leukemia patients. Leukemia 2010;24:1388-1392.
6. Hambach L, Aghai Z, Pool J, Kroger N, Goulmy E. Peptide length extension skews the minor HA-1 antigen presentation toward activated dendritic cells but reduces its presentation efficiency. J Immunol 2010;185:4582-4589.
7. Mommaas B, Van Halteren AG, Pool J et al. Adult and cord blood T cells can acquire HA-1 specificity through HA-1 T-cell receptor gene transfer. Haematologica 2005;90:1415-1421.
8. van Loenen MM, de BR, Hagedoorn RS, van Egmond EH, Falkenburg JH, Heemskerk MH. Optimization of the HA-1-specific T-cell receptor for gene therapy of hematologic malignancies. Haematologica 2011;96:477-481.
9. Goulmy E, Pool J, van den Elsen PJ. Interindividual conservation of T-cell receptor beta chain variable regions by minor histocompatibility antigen-specific HLA-A*0201- restricted cytotoxic T-cell clones. Blood 1995;85:2478-2481.
10. Verdijk RM, Mutis T, Wilke M et al. Exclusive TCRVbeta chain usage of ex vivo generated minor Histocompatibility antigen HA-1 specific cytotoxic T cells: implications for monitoring of immunotherapy of leukemia by TCRBV spectratyping. Hematol J 2002;3:271-275.
11. Cai J, Lee J, Jankowska-Gan E et al. Minor H Antigen HA-1-specific Regulator and Effector CD8+ T Cells, and HA-1 Microchimerism, in Allograft Tolerance. J Exp Med 2004;199:1017-1023.
12. van Halteren AGS, Jankowska-Gan E, Joosten A et al. Naturally acquired tolerance and sensitization to minor histocompatibility antigens in healthy family members. Blood 2009;114:2263-2272.
13. Mutis T, Verdijk R, Schrama E, Esendam B, Brand A, Goulmy E. Feasibility of immunotherapy of relapsed leukemia with ex vivo- generated cytotoxic T lymphocytes specific for hematopoietic system- restricted minor histocompatibility antigens. Blood 1999;93:2336-2341.
14. Mutis T, Gillespie G, Schrama E, Falkenburg JH, Moss P, Goulmy E. Tetrameric HLA class I-minor histocompatibility antigen peptide complexes demonstrate minor histocompatibility antigen-specific cytotoxic T lymphocytes in patients with graft-versus-host disease. Nat Med 1999;5:839-842.
15. Oosten LE, Blokland E, Van Halteren AG et al. Artificial antigen-presenting constructs efficiently stimulate minor histocompatibility antigen-specific cytotoxic T lymphocytes. Blood 2004;104:224-226.
16. Gillespie G, Mutis T, Schrama E et al. HLA class I-minor histocompatibility antigen tetramers select cytotoxic T cells with high avidity to the natural ligand. The Hematology Journal 2001;1:403-410.
17. Hambach L, Nijmeijer BA, Aghai Z et al. Human cytotoxic T lymphocytes specific for a single minor histocompatibility antigen HA-1 are effective against human lymphoblastic leukaemia in NOD/scid mice. Leukemia 2006;20:371-374.