LB-ARHGDB-1R as a novel minor histocompatibility antigen for therapeutic application

In HLA-matched allogeneic hematopoietic stem cell transplantation (SCT), donor T cells can mediate graft-versus-leukemia/lymphoma (GvL) reactivity and graft-versus-host disease (GvHD) by recognition of minor histocompatibility antigens (MiHA). Only a minority of MiHA shows hematopoiesis-restricted expression, and donor T cells for these MiHA may induce beneficial GvL reactivity without GvHD. The number of well-characterized MiHA with therapeutic relevance based on hematopoiesis-restricted expression remains limited and only 25% and 40% of recipients transplanted with grafts from sibling and unrelated donors, respectively, are eligible for therapies targeting known hematopoietic MiHA. Thus, in order to increase the efficacy and applicability of cellular therapy for selective GvL induction, more hematopoiesis-restricted MiHA with balanced population frequencies in common HLA molecules must be identified. Here, we investigated the therapeutic significance of a MiHA encoded by ARHGDB. We demonstrated hematopoiesis-restricted gene expression with the exception of intermediate mRNA expression in endothelial cells and showed that T cells recognized LB-ARHGDB-1R presented by HLA-B*07:02 on primary leukemic cells, but not on [interferon-gamma (IFN-γ)-treated] fibroblasts and keratinocytes. To evaluate potential toxicity against endothelial cells, we tested T cell recognition of LB-ARHGDB-1R on human umbilical vein endothelial cells (HUVEC) and found only limited reactivity under inflammatory conditions. Furthermore, we demonstrated in vivo targeting of LB-ARHGDB-1R in eight out of ten patients who were screened for post-transplant specific T-cell responses. In one patient with relapsed lymphoma, high T-cell frequencies were induced after donor lymphocyte infusion (DLI), coinciding with long-lasting anti-lymphoma immunity without GvHD. Our data thus support the relevance of LB-ARHGDB-1R as a therapeutic target with the potential to induce selective GvL reactivity.

We previously demonstrated that CD8 T cells specific for a MiHA (LB-ARHGDB-1R) encoded by the ARHGDB gene were induced in a patient with myelodysplastic syndrome who responded to DLI after HLA-matched allogeneic SCT. LB-ARHGDB-1R is translated from the normal ARHGDB transcript (NM_001175) in an alternative reading frame. Since ARHGDB has been described to be expressed in hematopoietic cells, we investigated the therapeutic value of LB-ARHGDB-1R to stimulate GvL reactivity after allogeneic SCT without GvHD. We first examined ARHGDB expression by microarray gene expression analysis using Illumina HT-12 v3.0 (BeadChips®) and compared gene expression between (malignant) hematopoietic and non-hematopoietic cells, which were cultured in the absence or presence of IFN-γ to mimic a state of inflammation. ARHGDB showed strong overexpression in the majority of (malignant) hematopoietic versus (IFN-γ pre-treated) non-hematopoietic cells. The ARHGDB expression profile was comparable to the strictly hematopoietic HMHA1 and MYO1G (Figure 1A and data not shown).

Next, we investigated T-cell recognition of different leukemic samples and both primary fibroblasts and keratinocytes cultured from skin biopsies in the absence or presence of IFN-γ. Samples were collected from patients and healthy individuals after approval from the Leiden or Radboud UMC Institutional Review Board and informed consent according to the Declaration of Helsinki. Recognition of (non-)hematopoietic cell types by LB-ARHGDB-1R-specific T cells was measured by IFN-γ enzyme-linked immunosorbent assay (ELISA) after overnight co-incubation. The data confirmed hematopoiesis-restricted T-cell recognition of LB-ARHGDB-1R in HLA-B*07:02 on all MiHA-positive leukemic cells, but not on (IFN-γ pre-treated) fibroblasts and keratinocytes (Figure 1B). T cells for LB-ARHGDB-1R were also shown to recognize healthy hematopoietic cell types, including Epstein-Barr virus-infected (EBV) B cells, phytohemagglutinin-stimulated T cells and dendritic cells (Figure 1B and data not shown), indicating that alternative translation of LB-ARHGDB-1R is not restricted to malignant cells. T cells for LB-ARHGDB-1R also showed specific lysis of patient’s, but not donor’s, EBV-B cells and specific lysis of an acute lymphoblastic leukemia sample (ALL #1) in a 10 h 51Cr-release assay (Figure 1C). Lysis of other acute lymphoblastic and myeloid leukemia samples was not detected by 51Cr-release, but could be measured after 48 h co-incubation in a flow cytometry-based cytotoxicity assay, while (IFN-γ pre-treated) patient’s fibroblasts were not lysed (Figure 1D and data not shown). The data showed that T cells for LB-ARHGDB-1R can specifically lyse hematological malignancies of different origins.

In addition to hematopoietic cells, ARHGDB can be expressed in endothelial cells. Therefore, we addressed potential toxicity and measured T-cell reactivity against endothelial cells. We confirmed intermediate ARHGDB gene expression in HUVEC under both steady-state and inflammatory conditions by microarray gene expression analysis (Online Supplementary Figure S1A, upper panel). Increased ARHGDB mRNA expression in HUVEC as compared to fibroblasts was also detectable by quantitative polymerase chain reaction analysis (see Online Supplementary Methods) (Online Supplementary Figure S1A, lower panel). To investigate whether gene expression in HUVEC can lead to T-cell recognition, we measured reactivity against two LB-ARHGDB-1R-positive HUVEC samples by IFN-γ ELISA. T cells for LB-ARHGDB-1R were only capable of recognizing one HUVEC sample (#2) after IFN-γ pre-treatment, which is known to up-regulate HLA, co-stimulatory and adhesion molecules and to increase the antigen processing and presentation capacity (Online Supplementary Figure S1B). However, recognition of HUVEC #2 was low compared to EBV-B cells and (IFN-γ pre-treated) HUVEC #1 was not or hardly recognized by specific T cells. Altogether, we demonstrate that ARHGDB gene expression in HUVEC leads to low surface presentation of LB-ARHGDB-1R that triggers only minimal T-cell reactivity under inflammatory conditions. Our results thus support the value of LB-ARHGDB-1R as a target for T-cell therapy to selectively augment GvL reactivity after allogeneic SCT with a limited risk of GvHD. Finally, we determined the in vivo immunogenicity of LB-ARHGDB-1R and investigated its relevance as a T-cell target in clinical responses after allogeneic SCT. The population frequency of LB-ARHGDB-1R in Caucasians is 77% (www.hapmap.org), resulting in a disparity rate, in which a LB-ARHGDB-1R-positive patient is transplanted with a negative donor, of 18%. HLA-B*07:02 is expressed in approximately 20% of Caucasians. In our cohort of 93 HLA-B*07:02 patient-donor pairs, 14 LB-ARHGDB-1R-positive patients were transplanted with a MiHA-negative donor and samples at relevant time points were available for ten patients.
Peripheral blood mononuclear cells were stained with APC- and PE-conjugated HLA-B*07:02 tetramers containing LB-ARHGDIB-1R directly *ex vivo* as well as after 7 days of *in vitro* peptide stimulation as previously described. Peripheral blood mononuclear cells obtained after allogeneic SCT (and DLI) were analyzed for tetramer-positive T cells, and LB-ARHGDIB-1R-specific T cells were detected in four patients *ex vivo* (Figure 2A) and in four additional patients after *in vitro* peptide stimulation (Figure 2B), resulting in eight positive patients (80%) out of a total of ten.

All patients were treated with partial T-cell-depleted allogeneic SCT followed by at least 4 months of immunosuppression with cyclosporine A as GVHD prophylaxis. Six of ten patients received DLI after allogeneic SCT (Online Supplementary Table S1). In three of six patients treated with DLI, tetramer-positive T cells were detected *ex vivo* at frequencies between 0.06-0.92%. Five patients...
received prophylactic DLI, and a clinical response against
(malignant) hematopoietic cells of patient origin could
not therefore be monitored. Patient #5 with relapsed fol-
licular lymphoma, which was confirmed by lymph node
biopsy, received therapeutic DLI that in the absence of
additional (chemo)therapy induced a long-lasting com-
plete remission (>16 years) without any signs of GvHD.

T cells for LB-ARHGDIB-1R were measured in three
patients with GvHD after DLI (patients #2, 6 and 7). These
T cells were detectable ex vivo (0.92%) in patient #2 and after in vitro stimulation
in the other two patients. Although it cannot be excluded
that T cells for LB-ARHGDIB-1R may have contributed
to GvHD in these patients, we consider it more likely
that T cells with other specificities mediated GvHD, since
we previously demonstrated that a variety of MiHA are
often targeted in patients with GvHD, and that the majority of these MiHA are ubiquitously expressed on
(non-)hematopoietic tissues. This is further supported by
the observation that T cells for LB-ARHGDIB-1R were
also measured ex vivo (0.13%) in patient #5. Induction of
tetramer-positive T cells in this patient 2 months after
DLI coincided with long-lasting GvL reactivity without
GvHD. Dynamic analysis of LB-ARHGDIB-1R tetramer-
positive T cells in this patient demonstrated high fre-
quencies not only after DLI but also within the first
weeks after allogeneic SCT during immunosuppression
with cyclosporine A (Figure 2C). Although the long-last-
ing GvL response in patient #5 suggests that LB-
ARHGDIB-1R-specific T cells are capable of mediating
strong anti-tumor immunity, T cells with specificities
other than LB-ARHGDIB-1R may also be involved in
the therapeutic effect of DLI. Systemic toxicity as a result
of vascular damage has not been observed in any of the
patients with circulating LB-ARHGDIB-1R-specific
T cells. Thus, clinical observations support the therapeu-
tic value of LB-ARHGDIB-1R and do not show evidence
for specific attack of endothelial cells as might be suggest-
ed based on detectable ARHGDIB gene expression and
low T-cell recognition of endothelial cells in vitro.

In conclusion, our data support the relevance of LB-
ARHGDIB-1R as highly immunogenic and
hematopoiesis-restricted MiHA with the potential to
shift the delicate balance between GvL reactivity and
GvHD in favor of desired anti-tumor reactivity. At the
Radboud UMC, we have started a clinical trial in which
transplanted patients are vaccinated with donor dendritic
cells loaded with mRNA and included ARHGDIB as one
of the transcripts for hematopoiesis-restricted MiHA
(Dutch Trial Registry #NTR4128). Future clinical data

Figure 2. LB-ARHGDIB-1R as an
immunogenic target with rele-
vance for GvL reactivity. Peripheral
blood mononuclear cells from ten
LB-ARHGDIB-1R and HLA-B*07:02
positive patients transplanted with
HLA-mismatched negative donors
were screened for LB-ARHGDIB-1R-
specific CD8 T cells directly
ex vivo as well as after 1 week of in vitro
peptide stimulation (IVS). (A)
Patients with detectable LB-
ARHGDIB-1R tetramer-positive
T-cells ex vivo. Numbers indicate
the percentage of CD8 T cells that
are positive for both LB-ARHGDIB-
1R tetramers (PE and APC) in the
Sytox blue neg, CD8 pos, CD4 neg,
CD14 neg, CD16 neg, and CD19 neg
T-cell population. (B) Dot plots of
patients with detectable
LB-ARHGDIB-1R tetramer-positive
T-cells ex vivo. Numbers indicate
the percentage of CD8 T cells that
are positive for both LB-ARHGDIB-
1R tetramers (PE and APC). (C) Ex vivo frequen-
cies of LB-ARHGDIB-1R specific T
cells and clinical response in
patient #5 who was treated with
DLI in the absence of additional
(chemo)therapy for relapsed lym-
phoma after partial T-cell-depleted allogeneic SCT.
will, therefore, provide definite evidence of whether T cells for LB-ARHGDB-1R are capable of inducing selective GVL responses. In addition to hematopoietic cells, ARHGDB has been reported to be expressed in several solid tumors correlating with advanced tumor stage and metastatic potential. As such, LB-ARHGDB-1R may have broad value as a target for T-cell therapy to treat hematologic malignancies and solid tumors after allogeneic SCT.

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doi:10.3324/haematol.2015.125021

Acknowledgments: the authors would like to thank Cynthia Kramer (Radboud UMC) for tetramer analyses and cell culture, and Martijn Dane (Department of Nephrology and the Einthoven Laboratory of Experimental Vascular Medicine, Leiden University Medical Center, Leiden, The Netherlands) for isolation of HUVEC

Funding: this work was supported by the Dutch Cancer Society (UL 2010-4748) and ZonMW (grant 95103004).

Key words: LB-ARHGDB-IR, minor histocompatibility antigen, hematopoiesis-restricted expression.

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

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