LB-ARHGDB1-1R 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 ARHGDB1. We demonstrated hematopoiesis-restricted gene expression with the exception of intermediate mRNA expression in endothelial cells and showed that T cells recognized LB-ARHGDB1-1R 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-ARHGDB1-1R on human umbilical vein endothelial cells (HUVEC) and found only limited reactivity under inflammatory conditions. Furthermore, we demonstrated intermediate MiHA with therapeutic relevance based on hematopoiesis-restricted expression by microarray gene expression analysis (Online Supplementary Figure S1A, upper panel). Increased ARHGDB1 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-ARHGDB1-1R-positive HUVEC samples by IFN-γ ELISA. T cells for LB-ARHGDB1-1R 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 ARHGDB1 gene expression in HUVEC leads to low surface presentation of LB-ARHGDB1-1R that triggers only minimal T-cell reactivity under inflammatory conditions. Our results thus support the value of LB-ARHGDB1-1R as a target for T-cell therapy to selectively augment GvL reactivity after allogeneic SCT without GvHD. Finally, we determined the in vivo immunogenicity of LB-ARHGDB1-1R and investigated its relevance as a T-cell target in clinical responses after allogeneic SCT. The population frequency of LB-ARHGDB1-1R in Caucasians is 77% (www.hapmap.org), resulting in a disparity rate, in which a LB-ARHGDB1-1R-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-ARHGDB1-1R-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 \textit{ex vivo} as well as after 7 days of \textit{in vitro} peptide stimulation as previously described.\(^1\) 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 \textit{ex vivo} (Figure 2A) and in four additional patients after \textit{in vitro} peptide stimulation, 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 \textit{ex vivo} at frequencies between 0.06-0.92\%.

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received prophylactic DLI, and a clinical response against (malignant) hematopoietic cells of patient origin could not therefore be monitored. Patient #5 with relapsed follicular lymphoma, which was confirmed by lymph node biopsy, received therapeutic DLI that in the absence of additional (chemo)therapy induced a long-lasting complete remission (>16 years) without any signs of GvHD. T cells for LB-ARHGDB1-R were measured in three patients with GvHD after DLI (patients #2, 6 and 7). These T cells were detectable \textit{ex vivo} (0.92%) in patient #2 and after \textit{in vitro} stimulation in the other two patients. Although it cannot be excluded that T cells for LB-ARHGDB1-R 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,\textsuperscript{5,14} 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-ARHGDB1-R were also measured \textit{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-ARHGDB1-R tetramer-positive T cells in this patient demonstrated high frequencies not only after DLI but also within the first weeks after allogeneic SCT during immunosuppression with cyclosporine A (Figure 2C). Although the long-lasting GvL response in patient #5 suggests that LB-ARHGDB1-R-specific T cells are capable of mediating strong anti-tumor immunity, T cells with specificities other than LB-ARHGDB1-R 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-ARHGDB1-R-specific T cells. Thus, clinical observations support the therapeutic value of LB-ARHGDB1-R and do not show evidence for specific attack of endothelial cells as might be suggested based on detectable ARHGDB gene expression and low T-cell recognition of endothelial cells \textit{in vitro}.

In conclusion, our data support the relevance of LB-ARHGDB1-R 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 ARHGDB as one of the transcripts for hematopoiesis-restricted MiHA (Dutch Trial Registry \#NTR4128). Future clinical data

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\caption{LB-ARHGDB1-R as an immunogenic target with relevance for GvL reactivity. Peripheral blood mononuclear cells from ten LB-ARHGDB1-R and HLA-B*07:02 positive patients transplanted with HLA-matched negative donors were screened for LB-ARHGDB1-R-specific CD8 T cells directly \textit{ex vivo} as well as after 1 week of \textit{in vitro} peptide stimulation (IVS)\textsuperscript{13} (A) Patients with detectable LB-ARHGDB1-R tetramer-positive T-cells \textit{ex vivo}. Numbers indicate the percentage of CD8 T cells that are positive for both LB-ARHGDB1-R tetramers (PE and APC) in the Sytox blue\textsuperscript{-}, CD8\textsuperscript{+}, CD4\textsuperscript{-}, CD14\textsuperscript{-}, CD16\textsuperscript{-}, and CD19\textsuperscript{-} T-cell population. (B) Dot plots of patients with detectable LB-ARHGDB1-R tetramer-positive T-cells \textit{ex vivo}. Numbers indicate the percentage of CD8 T cells that are positive for both LB-ARHGDB1-R tetramers (PE and APC) in the Sytox blue\textsuperscript{-}, CD8\textsuperscript{+}, CD4\textsuperscript{-}, CD14\textsuperscript{-}, CD16\textsuperscript{-}, and CD19\textsuperscript{-} T-cell population. (C) Ex vivo frequencies of LB-ARHGDB1-R specific T cells and clinical response in patient #5 who was treated with DLI in the absence of additional (chemo) therapy for relapsed lymphoma after partial T-cell-depleted allogeneic SCT.}
\end{figure}
will, therefore, provide definite evidence of whether T cells for LB-ARHGDIB-1R are capable of inducing selective GvL responses. In addition to hematopoietic cells, ARHGDIB has been reported to be expressed in several solid tumors correlating with advanced tumor stage and metastatic potential. As such, LB-ARHGDIB-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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Key words: LB-ARHGDB-IR, minor histocompatibility antigen, hematopoiesis-restricted expression.

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References