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

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Supplemental methods:

Cell samples and culture

Human umbilical vein endothelial cells (HUVEC) were isolated from umbilical cords according to Jaffe *et al.*(1) using a cannula sized to fit the vein. HUVEC were cultured up to passage 4 in EGM-2 medium supplemented with the EGM-2 bullet kit (Lonza BioWhittaker, Basel, Switzerland) and refreshed every 3 days. Fibroblasts (FB), keratinocytes (KC) and HUVEC were cultured in the absence or presence of 200 IU/ml IFN-γ (Boehringer-Ingelheim, Ingelheim am Rhein, Germany) for 3 days. Retroviral transduction of the HLA-B*07:02 restriction allele was performed as described previously (2).

Quantitative RT-PCR

Expression of ARHGDIB mRNA was measured by quantitative real-time PCR using Taqman Universal Master Mix II, without UNG and Taqman gene expression assays for *ARHGDIB* (Hs00171288_m1) and normalized as a ratio of GAPDH (Hs99999905_m1) expression (all from Applied Biosystems, Life Technologies, Carlsbad, CA, USA). Amplifications were started with 10 minutes at 95°C, followed by 50 cycles of 15 seconds for denaturing at 95°C, 30 seconds of annealing at 60°C, and 30 seconds extension at again 60°C.

Supplemental results:

To investigate whether gene expression in HUVEC leads to surface presentation of LB-ARHGDIB-1R at levels that can be recognized by specific T-cells, we cultured LB-ARHGDIB-1R positive HUVEC #1 and #2 and measured T-cell recognition by IFN-γ ELISA after retroviral introduction of the HLA-B*07:02 restriction allele. Figure S1B shows that T-cells for LB-ARHGDIB-1R were only capable of recognizing HUVEC #2 after IFN-γ pre-treatment, which is known to enforce high expression of HLA, co-stimulatory and adhesion molecules as well as molecules involved in antigen processing and presentation (3, 4). However,

recognition of HUVEC #2 was low as compared to EBV-B cells and (IFN-γ pre-treated) HUVEC #1 was not or hardly recognized by specific T-cells. Peptide-loaded control experiments (Figure S1C) illustrated that HLA-B*07:02 surface expression on HUVEC was sufficient to mediate strong T-cell reactivity, which is in line with detection of these surface molecules by flow cytometry (Figure S1D). Altogether, the data demonstrate that *ARGHDIB* gene expression in HUVEC leads to low surface presentation of LB-ARHGDIB-1R at levels that trigger only minimal T-cell reactivity under inflammatory conditions. Our results thus support the value of LB-ARHGDIB-1R as target for T-cell therapy to selectively augment GvL reactivity after alloSCT with a limited risk for GvHD.

References:

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Figure S1: LB-ARHGDIB-1R as target with potential toxicity for endothelial cells

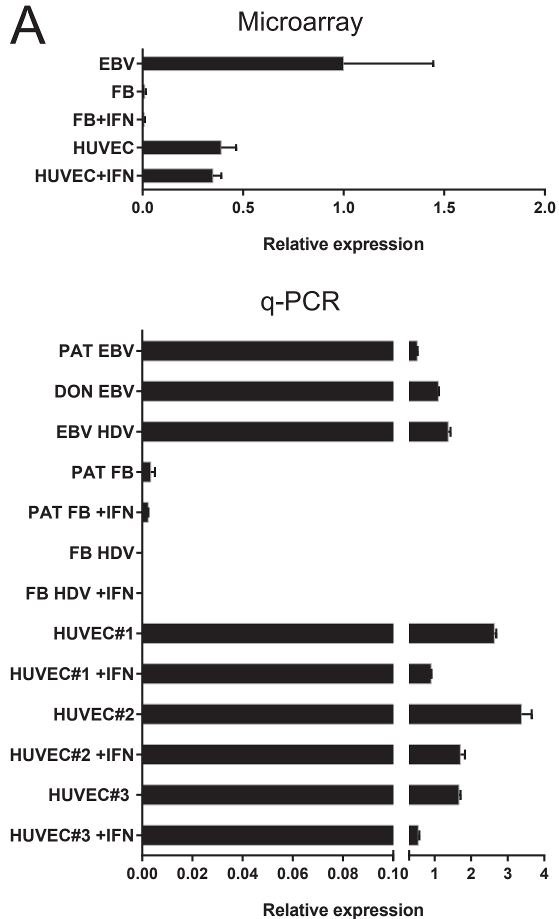
(A) *ARHGDIB* gene expression in FB and HUVEC relative to EBV-B cells by microarray gene expression analysis (upper panel) and quantitative RT-PCR (q-PCR; lower panel). FB and HUVEC were cultured in the absence or presence of IFN-γ. Gene expression by q-PCR was corrected for GAPDH expression.

(B) Reactivity of LB-ARHGDIB-1R specific T cells (clone H10) against HUVEC by IFN-γ ELISA. LB-ARHGDIB-1R positive HUVEC #1 and #2 were retrovirally transduced with HLA-B*07:02 (B7td) and cultured in the absence (upper panel) or presence (lower panel) of IFN-γ. LB-ARHGDIB-1R negative HUVEC #3 expressing HLA-B*07:02 endogenously (B7pos) and after retroviral introduction (B7td) were included as negative controls. T cell reactivity was also measured against EBV-B cells and FB of patient origin expressing HLA-B*07:02 endogenously (PAT EBV and FB) as well as EBV-B cells and FB from patient HDV expressing HLA-B*07:02 after retroviral transduction (HDV EBV-B7td and FB-B7td). EBV-B cells of donor origin (DON EBV) and EBV-B cells from LB-ARHGDIB-1R negative patient MAE expressing HLA-B*07:02 after retroviral transduction (MAE EBV-B7td) were included as negative controls. In addition to LB-ARHGDIB-1R specific T cells (clone H10), T cells for LB-GEMIN4-1V (clone Z1) and allo-HLA-A*02:01 (clone HSS12) were included. Genotyping results (+ or -) for SNPs encoding LB-ARHGDIB-1R (filled bars), LB-GEMIN4-1V (grey bars) and HLA-A*02:01 (open bars) are shown. Mean release of IFN-γ of duplicate wells is shown.

(C) LB-ARHGDIB-1R negative MAE EBV-B7td as well as LB-ARHGDIB-1R positive HDV FB-B7td and HUVEC #2-B7td were cultured in the absence of IFN-γ and pulsed with exogenous LB-ARHGDIB-1R at titrated peptide concentrations. These cells were subsequently compared for their capacity to stimulate T cells for LB-ARHGDIB-1R (clone H10) by IFN-γ ELISA (left panel). In addition, LB-ARHGDIB-1R negative HUVEC #3 B7pos and B7td were cultured in the absence of IFN-γ and compared for their stimulatory capacity (middle panel) as well as LB-ARHGDIB-1R positive HUVEC #2 B7td cultured in the absence or presence of IFN-γ (right panel). Mean release of IFN-γ of duplicate wells is shown.

(D) HLA-B*07:02 surface expression on EBV-B, FB and HUVEC B7pos or B7td by flow cytometry. FB and HUVEC were cultured in the presence of IFN-γ and stained with a PE-labeled antibody against HLA-B*07:02 (clone BB7.1). HLA-B*07:02 surface expression is shown by dark grey histograms. Light grey histograms represent non-stained EBV-B, FB and HUVEC and open histograms indicate antibody staining of EBV-B, FB and HUVEC that are negative for HLA-B*07:02.

Figure S1



B

without IFN-γ

with IFN-γ

clone H10 (LB-ARHGDIB-1R)

clone Z1 (LB-GEMIN4-1V)

clone HSS12 (Allo-HLA-A*02:01)

IFN-γ production ng/mL

0.0 0.5 1.0 1.5

LB-ARHGDIB-1R: + + + + - -

LB-GEMIN4-1V: - + - + + +

HLA-A*02:01: + - + - - -

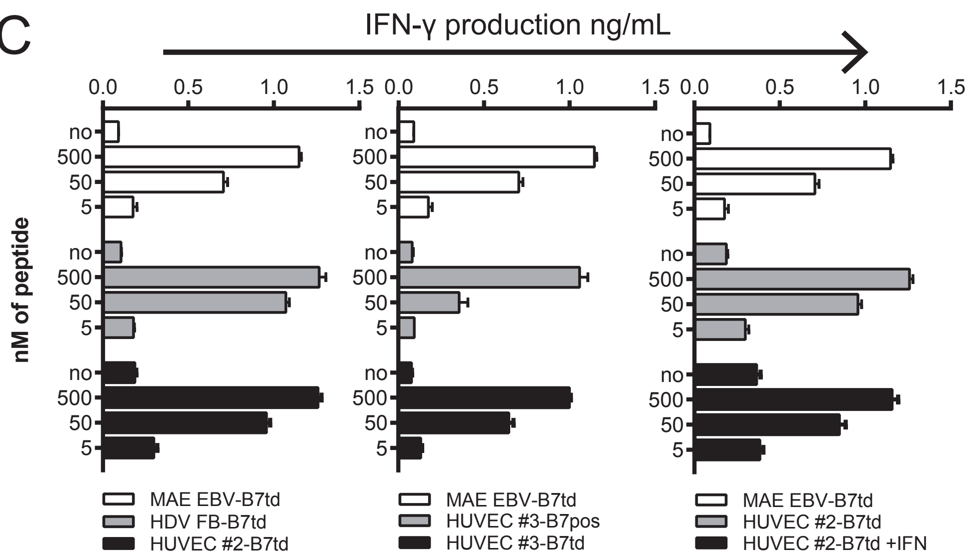
PAT FB | FB HDV B7td | HUVEC#1 B7td | HUVEC#2 B7td | HUVEC#3 B7pos | HUVEC#3 B7td

LB-ARHGDIB-1R: + - + -

LB-GEMIN4-1V: - - + -

HLA-A*02:01: + + - +

PAT EBV | DON EBV | HDV EBV B7td | MAE EBV B7td



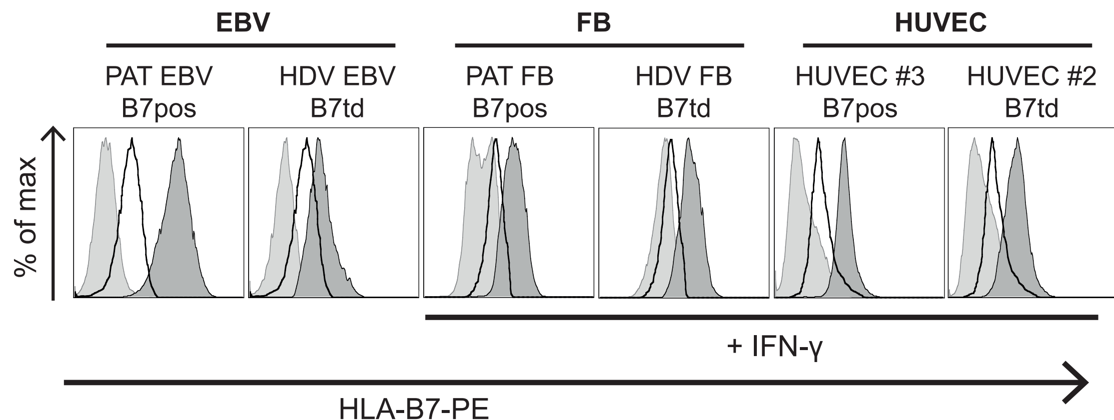


Table S1: Clinical data patients

patient	sex	diagnosis[a]	sample[b]	T cell response		acute GvHD after SCT (<100 days)[c]	chronic GvHD after SCT (>100 days)[d]	relapse after SCT	DLI after SCT[e]	GvHD after DLI[f]	total FU (years)	disease status
				ex vivo	*in vitro* stimulation							
1	F	AML-M2	6 m post-DLI	0.06%	0.09%	grade I (at d15)	no	no	prophylactic DLI (at 8 m)	no	10.0	remission
2	M	AML-M1	1 m post-DLI	0.92%	9.19%	no	no	no	prophylactic DLI (at 6 m)	grade II (at d46)	9.6	remission
3	F	AML-M2	3 m post-SCT	<0.01%	0.21%	no	limited (at d132)	no	no	-	6.4	remission
4	M	AML-M1	6 m post-SCT	0.07%	0.64%	grade I (at d23)	extensive (at d125)	no	no	-	13.0	remission
5	F	FL grade 2	2 m post-DLI	0.13%	1.31%	no	no	yes (at 22 m)	therapeutic DLI (at 24 m)	no	19.6	remission
6	F	AML-M5	6 m post-DLI	<0.01%	0.10%	no	no	no	prophylactic DLI (at 4 m)	extensive (at d372)	2.5	deceased due to GVHD
7	M	pro-B ALL	2 m post-DLI	<0.01%	0.02%	no	no	no	prophylactic DLI (at 6 m)	grade IV (at d69)	13.0	remission
8	F	AML	52 m post-DLI	<0.01%	0.18%	no	no	no	prophylactic DLI (at 8 m)	no	7.1	remission
9	F	AML-M2	3 m post-SCT	no	no	grade I (at d13)	no	no	no	-	18	remission
10	M	CLL	2 m post-SCT	no	no	grade III (at d26)	limited (at d149)	no	no	-	6.3	remission

[a] Diagnosis according to WHO classification. AML: acute myeloid leukemia; FL: follicular lymphoma; ALL: acute lymphoblastic leukemia, CLL: chronic lymphocytic leukemia, MDS: myelodysplastic syndrome.

[b] Peripheral blood samples for T-cell analysis have been collected at different time points after SCT or DLI. Time points are indicated in months (m) post-SCT or post-DLI.

[c] Acute GvHD was graded according to the criteria of Przepiorka et al. (Bone Marrow Transplant 1995). The time of diagnosis is indicated in days (d) after SCT.

[d] Chronic GvHD was classified according to the revised Seattle criteria of Lee et al. (Biol. Blood Marrow Transplant 2003). The time of diagnosis is indicated in days (d) after SCT.

[e] DLI was administered to prevent (prophylactic) or treat (therapeutic) relapse of the disease. The time of DLI is indicated in months (m) after SCT.

[f] GvHD was graded as in [3] (acute) or in [4] (chronic) The time of diagnosis is indicated in days (d) after DLI.