HLA class II restricted minor histocompatibility antigens - identification, processing and biology

Kremer, A.N.

4

HLA-DO regulates surface presentation of HLA-class-II restricted antigens on B-cell malignancies

Anita N. Kremer, Edith D. van der Meijden, M. Willy Honders, Margot J. Pont, Jelle J. Goeman, J.H. Frederik Falkenburg, Marieke Griffioen

Biology of Blood and Marrow transplantation, 2014 May;20(5):742-7.

ABSTRACT

Hematological malignancies often express surface HLA-class-II, making them attractive targets for $CD4^+$ T-cell therapy. We previously demonstrated that HLA-class-II ligands can be divided into DM-resistant and DM-sensitive antigens. In contrast to DM-resistant antigens, presentation of DM-sensitive antigens is suppressed by HLA-DM, but can be rescued by HLA-DO. We also showed that HLA-DO expression remains low in non-hematopoietic cells under inflammatory conditions, suggesting that DM-sensitive antigens may be ideal T-cell targets with a low risk for GvHD. Here, we demonstrated that B-cell malignancies often express HLA-DO and that levels are in particular high in chronic lymphocytic leukemia. Moreover, we showed that surface presentation of DM-sensitive antigens is regulated by HLA-DO, and that DM-sensitive antigens are relevant T-cell targets in B-cell malignancies and especially chronic lymphocytic leukemia. These data open the perspective to target HLA-class-II ligands with specific processing and presentation behavior for $CD4^+$ T-cell therapy of hematological malignancies.

INTRODUCTION

Allogeneic stem cell transplantation (aSCT) in the treatment of hematological malignancies is the most successful form of cellular immunotherapy.[1] The beneficial graft-versus-leukemia (GvL) effect is mediated by donor derived T-cells recognizing residual leukemic cells of the patient.[2] These T-cells, however, may also react against non-hematopoietic tissues of the patient, thereby inducing graft-versus-host disease (GvHD), the major cause of morbidity and mortality after aSCT.[3,4] A possible approach to shift the balance towards GvL is to exploit $CD4^+$ T-cells, since HLA-class-II is under normal conditions mainly expressed on cells of hematopoietic origin.[5,6] However, HLA-class-II can be upregulated on non-hematopoietic tissues under inflammatory conditions.

Recently, we identified a number of HLA-class-II restricted minor histocompatibility antigens[7,8] and analyzed their processing and presentation behavior. The data showed that HLA-class-II ligands can be divided into HLA-DM-resistant and -sensitive antigens.[9] DM-resistant antigens are presented on all HLA-class-II^{pos} cells, whereas presentation of DM-sensitive antigens is decreased by HLA-DM and restored by co-expression of its natural inhibitor HLA-DO. Interestingly, in contrast to HLA-DM, HLA-DO is hardly up-regulated by pro-inflammatory cytokines.[9,10] We therefore hypothesized that DM-sensitive antigens are inefficiently presented on non-hematopoietic cells under inflammatory conditions. This makes DM-sensitive antigens attractive targets for $CD4^+$ T-cell therapies with a low risk for GvHD. A prerequisite for selective GvL is, however, that DM-sensitive antigens are presented on leukemic cells.

We here investigated expression of HLA-DM and -DO in primary leukemic cells, and demonstrated that HLA-DO is often expressed in B-cell malignancies, in particular in chronic lymphocytic leukemia (CLL). We further confirmed the functional relevance of HLA-DO as regulator in surface presentation of DM-sensitive antigens on leukemic cells. Our data show that $CD4^+$ T-cells directed against DM-sensitive antigens are promising tools in aSCT, and emphasize the relevance of HLA-DO expression in cell types of different origins for optimal design of $CD4^+$ T-cell based immunotherapy.

MATERIALS AND METHODS

Cell samples and culture

Peripheral blood and bone marrow samples were obtained from patients and healthy individuals after approval by the Leiden University Medical Center Institutional Review Board and informed consent according to the Declaration of Helsinki. Mononuclear cells were isolated by Ficoll-Isopaque separation. T-cells, fibroblasts (FB), dendritic cells (DC) and cell line LB-ALL-SK were cultured as previously described.[9,11]

Microarray gene expression analysis

$CD14^+$ monocytes, $CD19^+$ B-cells, $CD3^+$ T-cells and $CD34^+$ hematopoietic stem cells (HSC) were isolated from (G-CSF mobilized) peripheral blood from healthy donors by flow-cytometry. Acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL) and multiple myeloma (MM) were isolated from patient samples by flow cytometry based on expression of CD33, CD34, CD19, CD19/CD5, and CD38 expression, respectively. FB were cultured from skin biopsies in the absence or presence of IFN-□ (100 IU/ml) for 4 days. Isolation of total RNA and microarray gene expression analysis was performed as described recently.[9]

Quantitative RT-PCR

Expression of HLA-DOA and HLA-DOB mRNA was measured by quantitative real-time RT-PCR using the Brilliant III Ultra Fast SYBR Green QPCR Mastermix (Agilent) and the following primers: DOA: 5'- TTTGCCCGCTTTGACCCGCA -3' and 5'- TCACCCGTGGAGGCACGTTG -3', DOB: 5'- AGGAGCAGACAGGCCGTGGA -3' and 5'- CCTCTGGTTGCACTTTTCTCCCCA -3'. Gene expression for DOA and DOB was normalized as ratio with 18S RNA expression. Amplifications were started with 10 min at 95°C, followed by 50 cycles of 30 sec for denaturing at 95°C, 30 sec of annealing at 60°C, and 30 sec extension at again 60°C. After amplification, specificity of the PCR products was confirmed by melting curve analysis.

Western blot analysis

Cells were lysed on ice in 1% triton-X lysis buffer containing protease inhibitors (Roche Diagnostics, Almere, The Netherlands). The sub-nuclear fraction was obtained by centrifugation at 10,000g for 30 min. SDS-Page was run on precast NuPage® Novex 10% Bis-Tris Mini gels (Life Technologies, Bleiswijk, The Netherlands) for 35 min. at 30V and blotted on PVDF membranes. HLA-DOB expression was detected by mouse monoclonal antibody s-69739 (Santa Cruz Biotechnology, Dallas, Texas 75220) and biotinylated goat anti-mouse IgG (Life Technologies).

Antigen presentation assays

Stimulator cells ($3x10^4$ cells/well) and $CD4^+$ T-cells ($5x10^3$ cells/well) were overnight co-incubated at 37°C in 96-well plates. IFN-γ release was measured by ELISA (Sanquin, Amsterdam, The Netherlands). As stimulator cells, Raji and LB-ALL-SK cell lines and primary CLL and ALL cells were used. Raji and LB-ALL-SK were retrovirally transduced with HLA-DQA1*01:03/DQB1*06:03 and HLA-DOA/DOB as recently described.[9] In addition, Raji and LB-ALL-SK expressing HLA-DQA1*01:03/DQB1*06:03 were transduced with lentiviral pLKO.1-puro containing short hairpin RNA for HLA-DOA (TRCN0000057268) or scrambled shRNA (SHC002) from the MISSION library (Sigma-Aldrich, Zwijndrecht, The Netherlands).

Flow cytometry based cytotoxicity

Target cells ($1x10^4$ cells/well) and $CD4^+$ T-cells ($3x10^4$ cells/well) were overnight co-incubated at 37°C in 96-well plates. LB-PI4K2B-1S peptide was added at 1 µg/ml. Co-cultures were stained with PE-labeled anti-CD3, and analysed by flow cytometry after addition of a fixed number of flow-count beads and propidium iodide (PI) to allow quantitative measurement of viable (PI negative) cells. As target cells, FB transduced with HLA-DQA1*01:03/DQB1*06:03 were used after pre-treatment with IFN-γ (100 IU/ml) for 4 days.

RESULTS

We recently proposed that DM-sensitive antigens may be relevant targets for T-cells with a selective GvL effect and low risk for GvHD. To investigate whether DM-sensitive antigens are relevant targets for GvL, primary leukemic cells of different origins were collected and compared for expression of HLA-DM (DMA and DMB) and DO (DOA and DOB) with non-malignant hematopoietic cells by microarray gene expression analysis (Figure 1A). The data showed that primary leukemic cells (AML, CML, ALL and CLL) expressed high levels of HLA-DMA and DMB, except for two AML-M3 which were also low in HLA-class-II (data not shown). HLA-DMA and DMB expression was low in all HLA-class-II^{neg} samples, which included MM, T-cells and FB cultured in the absence of IFN-γ. Similar as HLA-DMA and DMB, HLA-DOA expression was low in HLA-class-II^{neg} samples, but significant expression was observed in HLA-class-II^{pos} AML, CML, ALL and CLL, except for 7 AML, which included the two AML-M3 with low HLA-class-II. In contrast to HLA-DMA, DMB and DOA, expression of HLA-DOB was not or hardly induced in FB by IFN-γ, and we previously demonstrated that DM-sensitive antigens are inefficiently presented on FB in an inflammatory environment due to low levels of induced HLA-DOB.[9] Remarkably, a strong difference in HLA-DOB expression was observed between myeloid and lymphatic malignancies, and especially CLL. Except for mature DC, all samples from myeloid origin showed low expression of HLA-DOB, whereas strong expression was observed in CLL and healthy B-cells. Variable HLA-DOB expression was observed in ALL samples. HLA-DOB expression was high in the majority of MM, but these cells were HLA-class-II^{neg} (data not shown). The strong difference in HLA-DOB expression between CLL and myeloid malignancies could be confirmed by quantitative RT-PCR (data not shown) and Western blot analysis (Figure 1B). Strong HLA-DOB protein expression was observed in all CLL, whereas expression could not be detected in AML (Figure 1B). ALL cells showed variable HLA-DOB expression also on protein levels. The slight up-regulation of HLA-DOB in FB cultured under inflammatory conditions, which was observed on mRNA level, did not translate into detectable protein expression on Western blot (Figure 1B). In conclusion, our data show that HLA-DO in its full heterodimer is expressed at high levels in primary CLL and a subgroup of ALL.

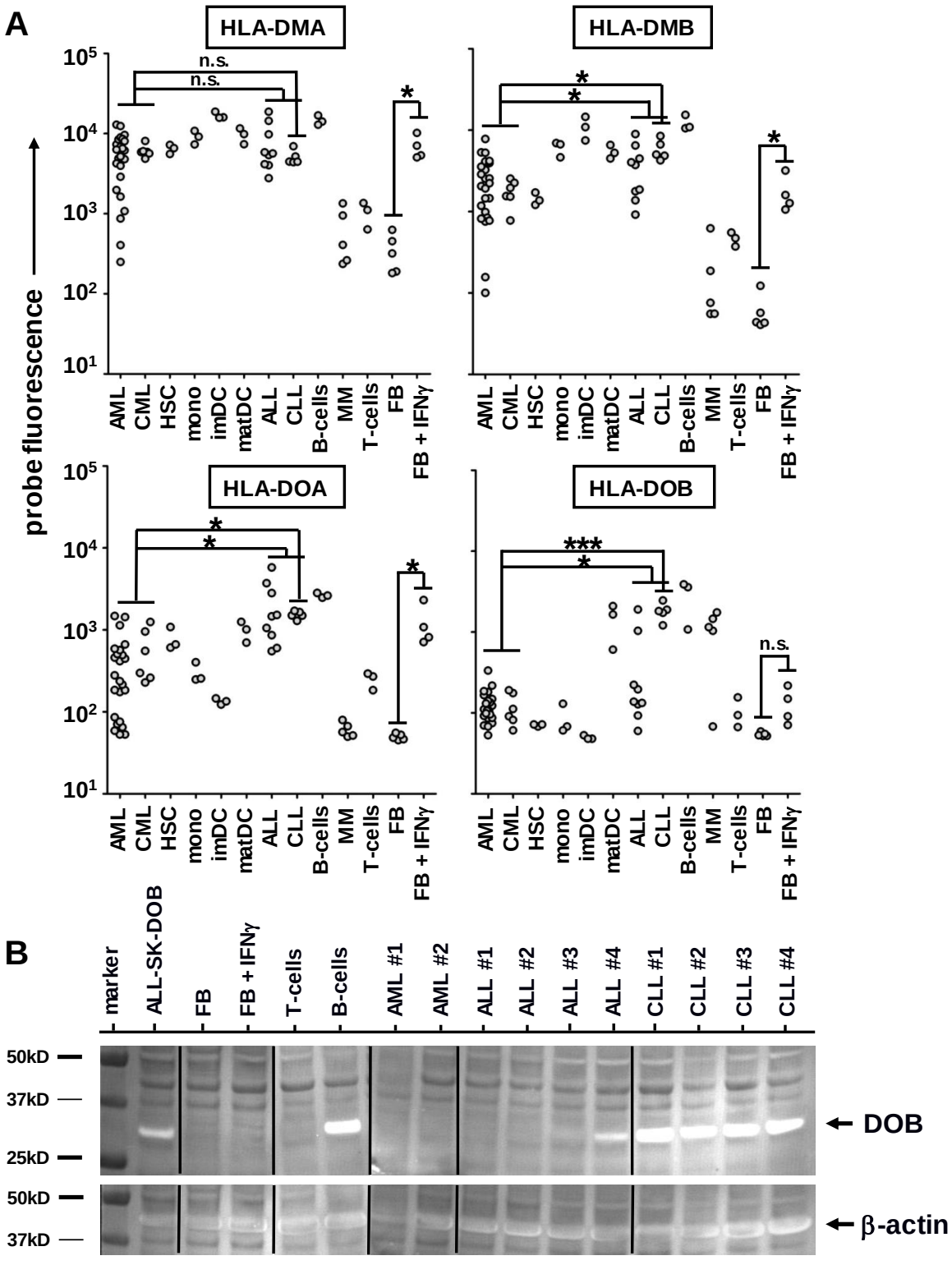
A
HLA-DMA
HLA-DMB
HLA-DOA
HLA-DOB
probe fluorescence
n.s.
AML
CML
HSC
mono
imDC
matDC
ALL
CLL
B-cells
MM
T-cells
FB
FB + IFNγ
B
marker
ALL-SK-DOB
AML #1
AML #2
ALL #1
ALL #2
ALL #3
ALL #4
CLL #1
CLL #2
CLL #3
CLL #4
50kD
37kD
25kD
DOB
β-actin

Figure 1. HLA-DM and DO expression in primary leukemic cells.
(A) The genes encoding the alpha and beta chains of HLA-DM (DMA and DMB) and HLA-DO (DOA and DOB) were analyzed by microarray gene expression analysis in acute myeloid leukemia (AML; n=18), chronic myeloid leukemia (CML; n=6), acute lymphoblastic leukemia (ALL; n=9), chronic lymphocytic leukemia (CLL; n=5) and multiple myeloma (MM; n=5) as well as in non-malignant hematopoietic stem cells (HSC; n=3), B-cells (n=3), T-cells (n=3), monocytes (n=3), monocyte-derived immature and mature dendritic cells (imDC and matDC; n=3), and non-hematopoietic fibroblasts (FB) cultured in the presence (n=4) or absence (n=5) of IFN-γ. Normalized probe fluorescence intensities are depicted for individual samples. Statistical analysis was performed using student's t-tests (n.s.; not significant; * $p<0.01$; ** $p<10-10$; *** $p<10-20$).
(B) HLA-DOB protein expression was measured by Western blot analysis in cell line LB-ALL-SK after retroviral transfer of the HLA-DOB gene, primary FB cultured in the absence or presence of IFN-γ, T-cells, B-cells, AML, ALL and CLL. Expression of β-actin is shown as loading control.

To investigate whether HLA-DO was functionally active in malignant B-cells and regulated surface presentation of DM-sensitive antigens, we first targeted HLA-DO in B-cell lymphoma cell line Raji and ALL cell line LB-ALL-SK by lentiviral introduction of HLA-DOA specific shRNA. Raji and LB-ALL-SK showed similar expression of HLA-DOA by microarray gene expression analysis, but expression of HLA-DOB was significantly different and HLA-DOB protein could only be detected in the Raji cell line (data not shown). Raji and LB-ALL-SK both endogenously expressed DM-sensitive antigen LB-PI4K2B-1S and the HLA-DQB1*06:03 restriction allele was retrovirally introduced. Quantitative RT-PCR confirmed a decrease in HLA-DOA mRNA expression upon introduction of specific, but not scrambled, shRNA (Figure S1A). T-cell experiments demonstrated that upon silencing of HLA-DOA, presentation of DM-sensitive antigen LB-PI4K2B-1S was significantly diminished, whereas presentation of DM-resistant control antigens were not affected (Figure 2A).

Next, we enforced HLA-DO expression in LB-ALL-SK by retroviral introduction of the HLA-DOA and DOB genes. Overexpression of HLA-DOA and DOB was confirmed by quantitative RT-PCR (Figure S1B) and Western blot analysis (Figure 1B). In T-cell experiments, presentation of LB-PI4K2B-1S was enhanced after HLA-DOB gene transfer, whereas presentation of a DM-resistant control antigen was not altered (Figure 2B).

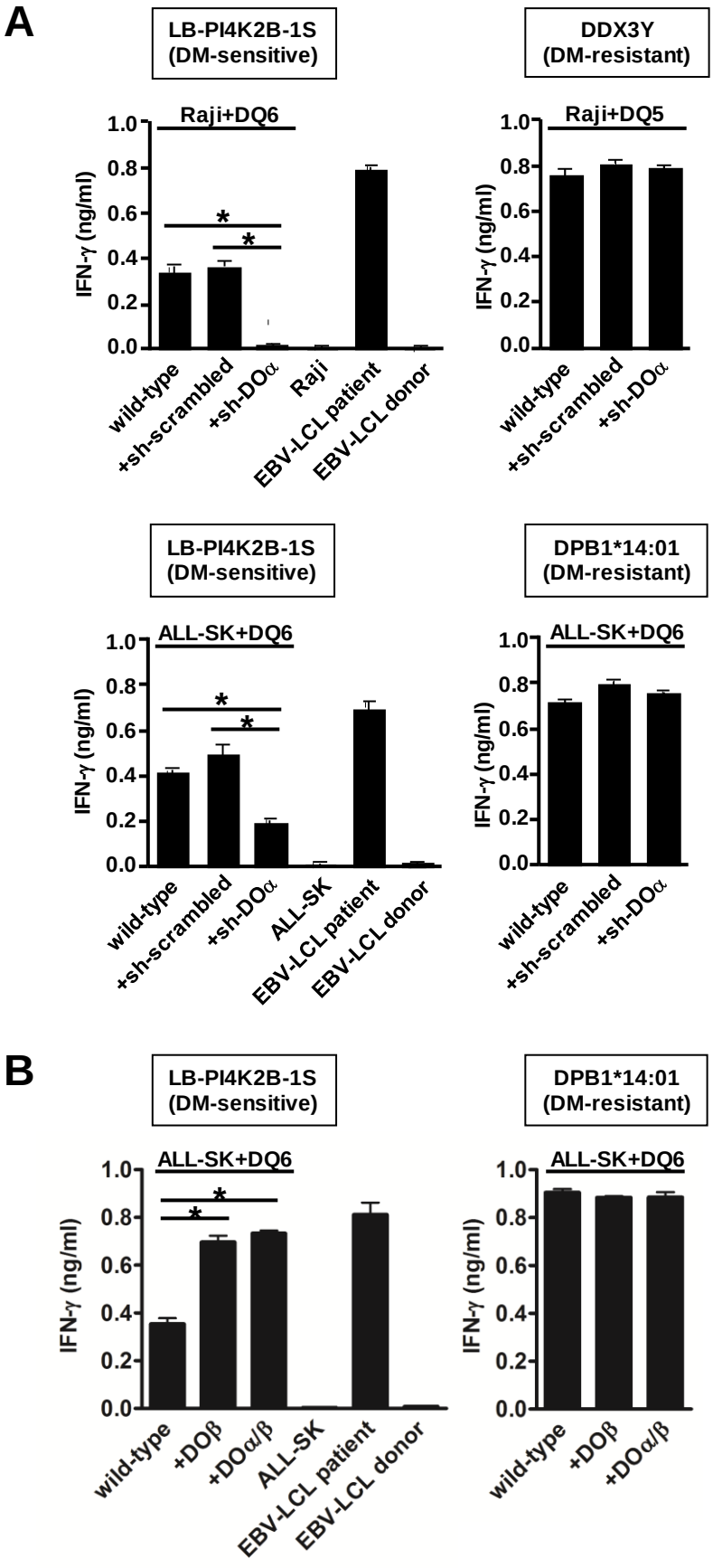
A
LB-PI4K2B-1S (DM-sensitive)
Raji+DQ6
IFN-γ (ng/ml)
1.0
0.8
0.6
0.4
0.2
0.0
*
*
wild-type
+sh-scrambled
+sh-DOα
Raji
EBV-LCL patient
EBV-LCL donor
DDX3Y (DM-resistant)
Raji+DQ5
wild-type
+sh-scrambled
+sh-DOα
LB-PI4K2B-1S (DM-sensitive)
ALL-SK+DQ6
wild-type
+sh-scrambled
+sh-DOα
ALL-SK
EBV-LCL patient
EBV-LCL donor
DPB1*14:01 (DM-resistant)
ALL-SK+DQ6
wild-type
+sh-scrambled
+sh-DOα
B
LB-PI4K2B-1S (DM-sensitive)
ALL-SK+DQ6
wild-type
+DOβ
+DOα/β
ALL-SK
EBV-LCL patient
EBV-LCL donor
DPB1*14:01 (DM-resistant)
ALL-SK+DQ6
wild-type
+DOβ
+DOα/β

Figure 2. HLA-DO regulates surface presentation of DM-sensitive antigens on B-cell malignancies.
(A) Silencing of HLA-DO by HLA-DOA specific shRNA reduces presentation of DM-sensitive antigen LB-PI4K2B-1S. B-cell lymphoma cell line Raji (upper) and ALL cell line LB-ALL-SK (lower) both endogenously express DM-sensitive antigen LB-PI4K2B-1S and the HLA-class-II restriction allele was expressed after retroviral transfer of the HLA-DQA1*01:03 and DQB1*06:03 genes. In a next step, Raji and LB-ALL-SK were transduced with lentiviral vectors containing silencing shRNA for HLA-DOA (TRCN0000057268) or scrambled control shRNA (SHC002) from the MISSION library. The T-cell clone for LB-PI4K2B-1S is an allo-reactive $CD4^+$ T-cell clone isolated from a patient with CML after HLA-matched alloSCT. For specificity of the T-cell clone, EBV-LCL from patient and donor were included as positive and negative controls, respectively. For the Raji cell line, a $CD4^+$ T-cell clone recognizing male-specific DM-resistant antigen DDX3Y was included as control, and the HLA-class-II restriction allele was expressed after retroviral transfer of the HLA-DQA1*01:01 and DQB1*05:01 genes. For LB-ALL-SK, an allo-reactive $CD4^+$ T-cell clone recognizing an unknown DM-resistant antigen in the endogenous HLA-DPB1*14:01 molecule was included. Mean release of IFN-γ in duplicate wells of one representative experiment is shown, but similar results were obtained in two other experiments. Significant differences in student's t-tests are indicated (* $p<0.05$).
(B) Enforced expression of HLA-DO by retroviral transfer of the HLA-DOB gene enhances presentation of DM-sensitive antigen LB-PI4K2B-1S. Cell line LB-ALL-SK expressing LB-PI4K2B-1S and its HLA-DQ restriction allele was retrovirally transduced with HLA-DOB alone or together with HLA-DOA. T-cell recognition of DM-sensitive antigen LB-PI4K2B-1S and the unknown DM-resistant antigen in HLA-DPB1*14:01 was measured by IFN-γ ELISA. Mean release of IFN-γ in duplicate wells of one representative experiment is shown, but similar results were obtained in two other experiments. Significant differences in student's t-tests are indicated (* $p<0.05$).

Finally, we investigated whether high expression of HLA-DOB in primary CLL as compared to ALL also translated into strong T-cell recognition of DM-sensitive antigens. Primary CLL and ALL samples were selected for expression of DM-sensitive antigen LB-PI4K2B-1S and an unknown DM-resistant antigen recognized by an allo-reactive HLA-DQB1*06:03-specific T-cell or for expression of DM-sensitive antigen LB-LY75-1K and DM-resistant antigen LB-PTK2B-1T and their relevant HLA-DR restriction alleles. The data showed that all leukemic samples were recognized by the T-cells, but that presentation of DM-sensitive antigens was superior on CLL as compared to ALL, whereas presentation of DM-resistant antigens was comparable (Figure 3A). Increased expression of HLA-DOA and DOB in these CLL as compared to ALL samples was confirmed by quantitative RT-PCR (Figure S1C). In conclusion, our data illustrate that HLA-DO regulates surface presentation of DM-sensitive antigens on B-cell malignancies, and that DM-sensitive antigens are relevant T-cell targets in particular for primary CLL which often express high HLA-DO.Since HLA-DO is not or hardly induced in non-hematopoietic cells by inflammatory cytokines[9,10], DM-sensitive antigens have been suggested to be T-cell targets

with a low risk for GvHD. To test the capacity of non-hematopoietic cells to present DM-sensitive antigens under inflammatory conditions, we retrovirally introduced HLA-DQB1*06:03 into skin derived FB endogenously expressing LB-PI4K2B-1S and tested T-cell mediated lysis of these FB after pre-treatment with IFN-γ and overnight co-incubation at an E:T ratio of 3:1. No or only low specific lysis of FB endogenously expressing LB-PI4K2B-1S was measured, whereas FB exogenously loaded with LB-PI4K2B-1S peptide showed strong specific lysis (Figure 3B). Our data illustrate that DM-sensitive antigens are inefficiently presented on non-hematopoietic cells under inflammatory conditions, and therefore support the therapeutic value of DM-sensitive antigens as T-cell targets with a low risk for GvHD.

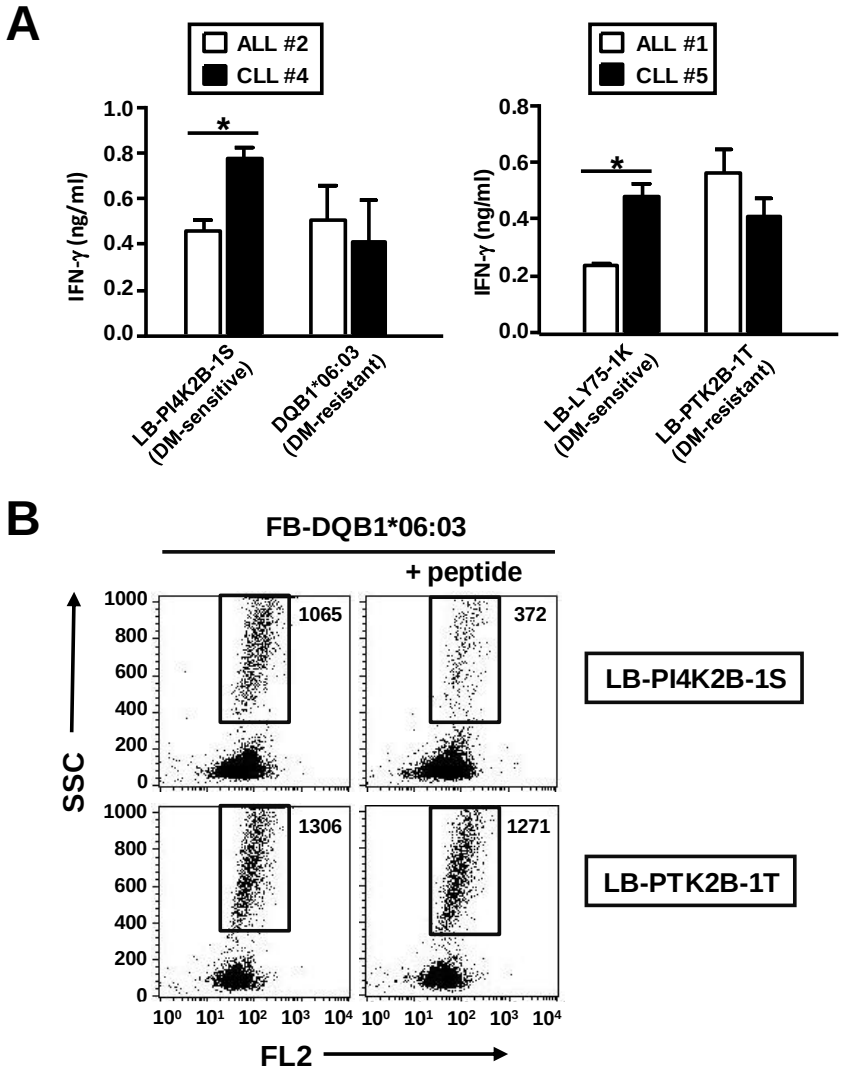

Figure 3. DM-sensitive antigens are efficiently presented on primary CLL, but not on skin derived FB under inflammatory conditions.
(A) DM-sensitive antigens are efficiently presented on high HLA-DO expressing CLL cells. Primary ALL and CLL were selected for expression of LB-PI4K2B-1S and HLA-DQB1*06:03 (left) or expression of LB-LY75-1K and LB-PTK2B-1T and their respective HLA-DRB3*01:01 and DRB1*13:01 restriction alleles (right). Primary ALL and CLL cells were sorted by flow cytometry and T-cell recognition of DM-sensitive antigens LB-PI4K2B-1S and LB-LY75-1K and DM-resistant antigen LB-PTK2B-1T and an unknown DM-resistant antigen recognized by an allo-reactive HLA-DQB1*06:03-specific T-cell clone was measured by IFN-γ ELISA. Mean release of IFN-γ of duplicate wells of two independent experiments are depicted.
(B) LB-PI4K2B-1S positive FB transduced with HLA-DQB1*06:03 were tested for lysis by LB-PI4K2B-1S specific T-cells after pre-treatment with IFN-γ. T-cells and FB were co-incubated overnight at an E:T ratio of 3:1, and numbers of viable (PI negative) FB were measured relative to a fixed number of flow-count beads in a quantitative flow-cytometry based cytotoxicity assay. T-cells for LB-PTK2B-1T were included as negative controls, since the FB did not express the HLA-DR restriction allele for these T-cells, and HLA-DQB1*06:03 transduced FB were exogenously loaded with LB-PI4K2B-1S peptide as positive control to confirm the lytic capacity of LB-PI4K2B-1S specific T-cells. Indicated is the side scatter and fluorescence as measured in FL2 for co-cultures of T-cells and FB. Fluorescence in FL2 for gated FB has been measured as a result of auto-fluorescence, whereas fluorescence in FL2 for remaining T-cells indicates positive staining with PE-labeled anti-CD3 antibody. Numbers of viable (PI negative) FB after overnight co-incubation with the indicated T-cells are shown. One representative of two experiments is shown.

DISCUSSION

We recently demonstrated that DM-sensitive antigens are inefficiently presented on non-hematopoietic cells under inflammatory conditions, suggesting that DM-sensitive antigens may be attractive targets to induce GvL with a reduced risk for GvHD. For GvL effect, however, DM-sensitive antigens should be presented on the surface of leukemic cells. We here show that HLA-DO is widely expressed in leukemic cells and that expression is sufficient for T-cell recognition of DM-sensitive antigens. Moreover, we demonstrate that HLA-DO expression is particularly high in primary CLL, and that these levels are functional as demonstrated by superior recognition of DM-sensitive antigens on CLL as compared to ALL.

HLA-DM has been described as chaperone that greatly accelerates peptide exchange in MHCII[12] and various studies have shown that HLA-DM induces release of peptides binding to MHCII with low affinity, resulting in a selection process that favors presentation

of high affinity peptides.[12-14] DM-resistant T-cell epitopes are therefore expected to bind with higher affinity to HLA-class-II than DM-sensitive T-cell epitopes. Our T-cell epitopes, however, are presented by different HLA-class-II alleles, each with its own binding characteristics and kinetics of HLA-DM catalysed peptide editing[14], and an accurate comparison of HLA-class-II binding affinity and half-lives between our DM-sensitive and DM-resistant T-cell epitopes is therefore not possible.

Our data show that HLA-DO regulates presentation of DM-sensitive antigens by HLA-class-II surface molecules on leukemic cells. Recently, Guce et al.[15] elucidated the crystal structure of the DO-DM complex, and demonstrated that DO and DM bind in a side-by-side arrangement similar to that proposed for MHC class II and DM. HLA-DO thus acts as a substrate mimic that tightly binds to DM, thereby preventing access to MHC class II. Guce et al.[15] also demonstrated that HLA-DO inhibits DM-mediated peptide release in a dose-dependent manner. Since DM is expressed at higher levels than DO, free DM and DO-DM complexes are both present, and the major mechanism for shifting their balance is via transcription of the DOB subunit[16]. This is also represented by our data showing constitutive expression of HLA-DMA, DMB and DOA in all HLA-class-II positive cells, whereas DOB expression is different between lymphoid and myeloid cells, showing high expression in healthy B-cells and CLL cells and variable expression in ALL. We further demonstrated that high HLA-DO expression indeed translates to T-cell recognition of DM-sensitive antigens, since (low-affinity) DM-sensitive peptides are efficiently presented on lymphatic leukemias and especially on CLL. Moreover, HLA-DOB is not expressed at detectable levels in skin-derived FB under inflammatory conditions, and T-cells for DM-sensitive antigens also failed to lyse cytokine pre-treated FB.

In conclusion, our data demonstrate and confirm the unique tissue distribution of HLA-DO and its function to inhibit DM-mediated peptide release in leukemic cells. These features of HLA-DO may be exploited for development of novel therapies in which $CD4^+$ T-cells for DM-sensitive antigens are used to induce GvL reactivity after aSCT with a low risk for GvHD.

ACKNOWLEDGEMENTS

The authors thank G. de Roo for technical assistance with flow cytometric isolation. This work has been supported by the Dutch Cancer Society (UL2008-4111), the German Research Foundation (DFG) (STU540/1-1) and the ELAN-Fond (12-04-05-1-Kremer) of the Friedrich-Alexander University Erlangen.

SUPPLEMENTARY INFORMATION

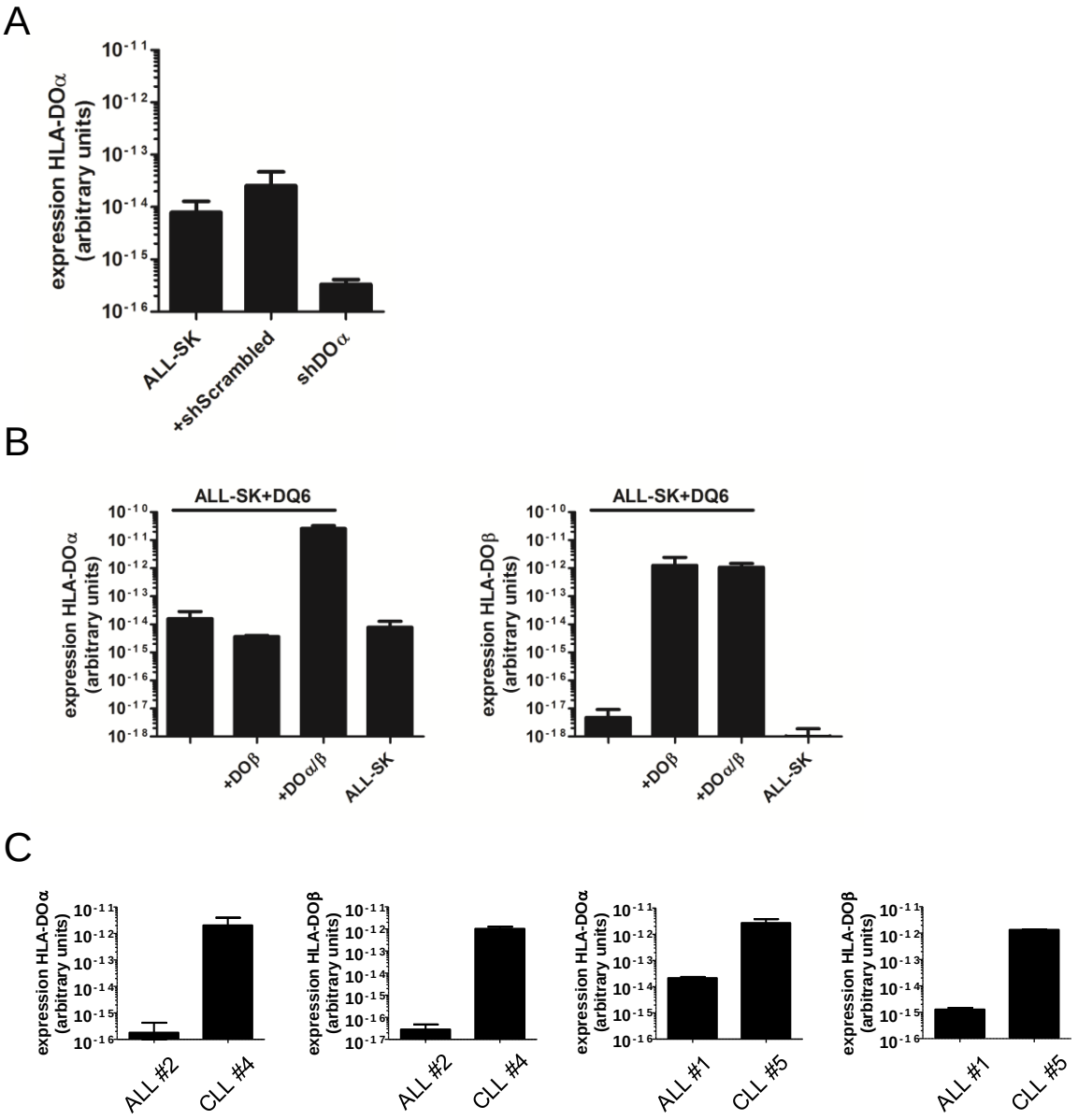

Figure S1. HLA-DOA and -DOB mRNA expression by quantitative RT-PCR.
(A) HLA-DOA mRNA expression corrected for expression of 18S RNA is shown for cell line LB-ALL-SK after transduction with scrambled and DOA specific short hairpin RNA.
(B) Relative mRNA expression of HLA-DOA (left) and -DOB (right) corrected for 18S RNA expression is shown for cell line LB-ALL-SK after retroviral transfer of HLA-DOB alone or together with HLA-DOA.
(C) HLA-DOA and -DOB mRNA expression corrected for 18S RNA for primary ALL and CLL.
In all graphs means and standard deviation of duplicate measurements of one representative experiment are shown.

REFERENCES

1. Copelan EA. Hematopoietic stem-cell transplantation. *N Engl J Med.* 2006;354:1813-1826.

2. Falkenburg JHF, Warren EH. Graft versus leukemia reactivity after allogeneic stem cell transplantation. *Biol Blood Marrow Transplant.* 2011;17:S33-S38.

3. Korngold R. Biology of graft-vs.-host disease. *Am J Pediatr Hematol Oncol.* 1993;15:18-27.

4. Ferrara JL, Levine JE, Reddy P, et al. Graft-versus-host disease. *Lancet.* 2009;373:1550-1561.

5. Meyer RG, Britten CM, Wehler D, et al. Prophylactic transfer of CD8-depleted donor lymphocytes after T-cell-depleted reduced-intensity transplantation. *Blood.* 2007;109:374-382.

6. Soiffer RJ, Alyea EP, Hochberg E, et al. Randomized trial of CD8+ T-cell depletion in the prevention of graft-versus-host disease associated with donor lymphocyte infusion. *Biol Blood Marrow Transplant.* 2002;8:625-632.

7. Griffioen M, van der Meijden ED, Slager EH, et al. Identification of phosphatidylinositol 4-kinase type II beta as HLA class II-restricted target in graft versus leukemia reactivity. *Proc Natl Acad Sci U S A.* 2008;105:3837-3842.

8. Stumpf AN, van der Meijden ED, van Bergen CAM, et al. Identification of 4 new HLA-DR-restricted minor histocompatibility antigens as hematopoietic targets in antitumor immunity. *Blood.* 2009;114:3684-3692.

9. Kremer AN, van der Meijden ED, Honders MW, et al. Endogenous HLA-class-II epitopes that are immunogenic in vivo show distinct behavior towards HLA-DM and its natural inhibitor HLA-DO. *Blood.* 2012;120:3246-3255.

10. Walter W, Scheuer C, Lingnau K, et al. H2-M, a facilitator of MHC class II peptide loading, and its negative modulator H2-O are differentially expressed in response to proinflammatory cytokines. *Immunogenetics.* 2000;51:794-804.

11. Nijmeijer BA, Szuhai K, Goselink HM, et al. Long-term culture of primary human lymphoblastic leukemia cells in the absence of serum or hematopoietic growth factors. *Exp Hematol.* 2009;37:376-385.

12. Pos W, Sethi DK, Wucherpfennig KW. Mechanisms of peptide repertoire selection by HLA-DM. *Trends Immunol.* 2013;34:495-501.

13. Yin L, Calvo-Calle JM, Dominguez-Amorocho O, et al.. HLA-DM constrains epitope selection in the human CD4 T cell response to vaccinia virus by favoring the presentation of peptides with longer HLA-DM-mediated half-lives. *J Immunol.* 2012;189:3983-3994.

14. Anders AK, Call MJ, Schulze MS, et al. HLA-DM captures partially empty HLA-DR molecules for catalyzed removal of peptide. *Nat Immunol.* 2011;12:54-61.

15. Guce AI, Mortimer SE, Yoon T, et al. HLA-DO acts as a substrate mimic to inhibit HLA-DM by a competitive mechanism. *Nat Struct Mol Biol.* 2013;20:90-98.

16. Denzin LK, Cresswell P. Sibling rivalry: competition between MHC class II family members inhibits immunity. *Nat Struct Mol Biol.* 2013;20:7-10