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Two Preferentially Expressed Proteins Protect Vascular Endothelial Cells from an Attack by Peptide-Specific CTL

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Vascular endothelial cells (EC) are an exposed tissue with intimate contact with circulating Ag-specific CTL. Experimental in vitro and clinical data suggested that endothelial cells present a different repertoire of MHC class I-restricted peptides compared with syngeneic leukocytes or epithelial cells. This endothelial-specific peptide repertoire might protect EC from CTL-mediated cell death. The HLA-A*02-restricted peptide profile of human EC and syngeneic B lymphoblastoid cells was biochemically analyzed and compared. For EC selective peptides, source protein expression, peptide binding affinity, and peptide–HLA-A*02 turnover were measured. The significance of abundant peptide presentation for target cell recognition by immunodominant CTL was tested by small interfering RNA treatment of EC to knock down the source proteins. High amounts of two peptides, $PTRF_{56–64}$ and $CD59_{106–114}$, were consistently detected in EC. This predominance of two endothelial peptides was explained by cell type-specific source protein expression that compensated for poor HLA-A*02 binding affinity and short half-live of peptide/HLA-A*02 complexes. Knocking down the source proteins containing the abundant endothelial peptide motifs led to a nearly 100-fold increase of surface expression of $SMCY_{311–319}$, an immunodominant minor histocompatibility Ag, as detected by cytotoxicity assays using $SMCY_{311–319}$-specific CTL. We conclude that EC express and present preferentially two distinct HLA-A*02-restricted peptides at extraordinary high levels. These abundant self-peptides may protect EC from CTL-mediated lysis by competing for HLA-A*02 binding sites with immunodominant scarcely expressed antigenic peptides. ***The Journal of Immunology*****, 2012, 188: 000–000.**

Human vascular endothelial cells (EC) form the inner lining of blood vessels and maintain organ homeostasis by several key functions; that is, undisturbed healthy EC prevent blood coagulation (1, 2), regulate vasomotion (3, 4). and actively participate in leukocyte trafficking (5, 6). EC are an exposed tissue that is in intimate contact with transmigrating effector lymphocytes in the course of immune responses (7). EC express histocompatibility Ags, that is, the molecular motifs recognized by the TCR (8), and therefore may serve as targets for Ag-specific effector lymphocytes. $CD8^+$ MHC class I-restricted CTL are activated by professional APC (e.g., dendritic cells) and differentiate to become effector CTL (9). In the course of allo- or autoimmune disorders, effector cells could rapidly eliminate EC when they shared the MHC class I-restricted peptide profile with dendritic cells. In contrast, when EC would present a completely different peptide profile than leukocyte-derived professional APC, they might by this mechanism escape CTL-mediated injury and death. A cell-specific peptide repertoire presented by endothelial MHC class I molecules would explain tissue or organ predilection of immune-mediated injury such as seen during graft–versus-host disease (10, 11). The hypothesis that EC present a different repertoire of MHC class I-restricted peptides was supported in the past by the following in vitro observations. First, the activation of human $CD8^+$ T lymphocytes by professional APC led to a subset of effector CTL that recognized and killed preferentially leukocyte-derived target cells but ignored EC from the same donor (12). Second, EC were poor targets for peptide-specific CTL due to an impaired capacity to present certain immunodominant Ags such as $SMCY_{311–319}$, a male-specific, HLA-A*02–restricted minor histocompatibility Ag, as efficiently as other target cell types (13). This cellular difference was not due to a general resistance of EC to CTL-mediated target cell lysis, to lack of costimulation, or to low MHC class I expression (13). Taken together, these observations are explained by a different repertoire of MHC class I ligands presented by EC. To prove that EC indeed present a different peptide repertoire compared with syngeneic leukocyte-derived B lymphoblastoid cells (BLC) serving in previous experiments as professional APC, we grew up sufficient numbers of EC and BLC from the same donor, isolated HLA-A*02–bound peptides from the two cell types, and characterized the resulting cell type-specific peptide profiles by mass spectrometry. By using this approach we identified HLA-A*02 ligands prevailing on EC, on BLC, or on both cells. Some endothelial peptides seemed to be specific for EC, that is, they were never identified so far on BLC. However, we found no novel or unique endothelial peptide species, but all were previously detected on other epithelial cell lines or in tumor tissues. Two peptides were present at dominant levels on EC. We further investigated mechanisms for this preferential peptide presentation on EC such as 1)

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Abbreviations used in this article: AUC, area under the curve; BLC, EBV-immortalized B lymphoblastoid cell; EC, vascular endothelial cell; FDR, false discovery rate; LC-MS, liquid chromatography/mass spectrometry; ΔMFI, change in mean fluorescence intensity; MFI, mean fluorescence intensity; MS, mass spectrometry; siRNA, small interfering RNA; TFA, trifluoroacetic acid.

expression levels of source proteins, 2) binding affinity to HLA-A*02, and 3) half-life of peptide/HLA-A*02 complexes. Finally, we used RNA interference to knock down the source proteins of the most abundant endothelial HLA-A*02–restricted peptides and tested the impact of this treatment on peptide presentation and on the susceptibility of EC and BLC to CTL-mediated killing.

Materials and Methods

Cell lines

All studies involving primary human cells were approved by the Ethical Review Board (Ethikkommission beider Basel). HUVEC (EC) were isolated from umbilical cords by enzymatic digestion as described (13). EC were cultured in complete medium 199 containing 20% FCS, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (all from Invitrogen Life Technologies, Carlsbad, CA), supplemented with fibroblast growth factors (20 ng/ml human acidic fibroblast growth factor and 20 ng/ml human basic fibroblast growth factor; both from PeproTech, London, U.K.) and heparin (0.2 mg/ml; Sigma-Aldrich, Saint Louis, MO). EBV-immortalized syngeneic BLC were grown from cord blood mononuclear cells (14). BLC were cultured in complete RPMI 1640 (Invitrogen Life Technologies) containing 10% FCS, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. The EBV-transformed B cell line JY, the TAP-deficient T2 cell line (both gifts from A. Cerny, Lugano, Switzerland), and the colon carcinoma cell line LS174 (a gift from G. Spagnoli, Basel, Switzerland) were cultured in complete RPMI 1640. For some experiments, $Flu_{58–66}$- and $SMCY_{311–319}$-specific, HLA-A*02–restricted CTL clones were used and kept in culture as previously described (13).

Quantification of HLA surface expression

EC were detached from cell culture flasks using Accutase (PAA Laboratories, Pasching, Austria) treatment for 3 min at 37°C and subsequently transferred to centrifugation tubes. BLC were resuspended by repeatedly pipetting and transferred to centrifugation tubes. Cells were filtered through a 70-μm nylon mesh to avoid clumping, washed twice with PBS, and counted. For each donor 5×10^5 EC or BLC were transferred to microtiter plates and washed twice with ice-cold FACS buffer (PBS, 2% FCS, 2 mM EDTA). Unless noted otherwise, all further steps were performed at 4°C. Cells were stained with HLA-A*02–specific BB7.2, HLA-A/B/C–specific W6/32, or respective isotype controls (BioLegend, San Diego, CA) at saturating conditions (10 μg/ml Ab diluted in FACS buffer). After two washing steps with FACS buffer, cells were stained with secondary FITC-conjugated anti mouse $F(ab')_2$ fragments (Dako) diluted 1:100 in FACS buffer. Quantification beads (Quifikit; Dako) were washed twice with FACS buffer and afterward stained with the same concentration of secondary Ab. Both cells and quantification beads were finally washed twice with FACS buffer and after addition of 5 μl 7-aminoactinomycin D analyzed on a FACSCanto II analyzer (BD Biosciences). For each sample 250,000 events were recorded and each experiment was performed in triplicate. Cells were gated on single cells based on FSC-A/FSC-H parameters and viable 7-aminoactinomycin D-negative cells using FlowJo FACS data analysis software (Tree Star). Generation of standard curve for quantification and subsequent calculation of surface expression were done according to manufacturer's instructions (Quifikit; Dako).

Isolation and sequence analysis of MHC class I-presented peptides

EC and syngeneic BLC from three different HLA-A*02–positive male donors were grown to large cell numbers and three independent peptide isolation experiments were performed. Per experiment, on average $5.2 \pm 0.9 \times 10^8$ EC were grown on 11,000 cm^2 (Falcon/BD Biosciences, San Jose, CA) over 45 d to confluence. For nine repetitive passages, cells were harvested by trypsin-EDTA (Invitrogen Life Technologies) digestion and reseeded finally on 64 gelatin-coated plates (Falcon/BD Biosciences, product no. 353025). At the final cell harvest, trypsinized cells were washed in complete medium 199, spun down, and 1.5 ml pelleted cells were snap frozen in liquid nitrogen. BLC were expanded as suspension cultures at an average density of 0.3×10^6 cells/ml to a final cell number of $1.9 \pm 0.5 \times 10^9$ cells per donor. BLC were also collected by centrifugation and briefly treated with ice-cold trypsin-EDTA to mimic the treatment of EC. Trypsin was neutralized with complete, ice-cold complete medium 199, the cells were spun down, and 1.5 ml pelleted cells were snap frozen in liquid nitrogen until the isolation and identification of MHC class I-bound peptides. HLA-presented peptides were obtained by immune precipitation of HLA molecules using an adapted protocol developed for solid tissue analysis (15). In brief, 1 vol lysis buffer containing PBS, 0.6% CHAPS, and complete protease inhibitor (Roche) was added to snap-frozen cell pellets and the samples were homogenized by intense shaking for 1 h at 4°C. Afterward, samples were sonicated and debris was removed by centrifugation and additional passing through a 0.2-μm filter (Sartorius, Göttingen, Germany). Immune precipitation of HLA-A*02 molecules was performed using the HLA-A*02–specific Ab BB7.2 covalently coupled to cyanogen bromide-activated Sepharose 4B (GE Healthcare, Freiburg, Germany; 40 mg Sepharose/mg Ab). MHC molecules and peptides were eluted in 0.1% trifluoroacetic acid (TFA) and peptides were isolated by ultrafiltration through a centricon 10 kDa cut-off membrane (Millipore, Schwalbach, Germany). For liquid chromatography/mass spectrometry (LC-MS) analysis, 20% of each sample was desalted and concentrated using C18 Zip Tips (Millipore) according to the manufacturer's instructions. Peptides were eluted in 50 μl 80% acetonitrile/0.1% TFA evaporated to nearly complete dryness by vacuum centrifugation and finally taken up in 5 μl loading solvent (2% acetonitrile, 98% H_2O, 0.1% TFA).

Nicotinylation of peptides

Chemical modification of synthetic peptides with deuterated nicotinic acid and peptide mixtures eluted from MHC precipitation with nicotinic acid were performed as previously described (16). In brief, peptides were first guanidinated in freshly prepared 2.5 M *O*-methylisourea hemisulfate at basic pH (>10.5) for 10 min at 65°C to protect lysine side chains. Reaction was terminated by addition of formic acid. Guanidinated peptides were then loaded on C18 Micro spin columns (Thermo Fisher Scientific) and modified by slowly passing 1 ml of a 2.2 mg/ml solution of either $^1H_4$- or $^2D_4$-nicotinoyloxysuccinimide (diluted in 50 mM phosphate buffer [pH 8.5]) over the column. After three washing steps with double distilled H_2O, potential tyrosine modifications were removed by passing 1 ml 50% hydroxylamine solution over the column. Following another three washing steps with double distilled H_2O, peptides were finally eluted in 80% acetonitrile/0.1% TFA and volumes were adjusted by vacuum centrifugation. Concentration of nicotinylated peptides was assessed using UV absorbance at 261 nm on a NanoDrop UV/Vis Spectrophotometer (Peqlab, Erlangen, Germany) against a standard curve generated from different concentrations of nicotinoyloxy-succinimide. For synthetic peptides, purity after deuterated nicotinylation was shown to be >80% using HPLC (Waters), and identity of peptides was confirmed using MS.

Nanoflow LC-MS/MS

MS was performed on an LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with a nanoelectron spray ion source coupled to an UltiMate 3000 RSLCnano UHPLC system (Dionex, Sunnyvale, CA). After injection samples were loaded on a 2 cm PepMap100 C18 Nano-Trap column (Dionex) within 10 min at a flow rate of 4 μl/min and 3% solvent B. Peptides were then separated on a 50 cm PepMap C18 column with a particle size of 2 μm (Dionex) running at 45°C with a flow rate of 300 nl/min and a gradient ranging from 3 to 30% solvent B within 140 min. For absolute quantification a different gradient was used ranging from 10 to 60% solvent B within 240 min (solvent A, H_2O, 0.02% formic acid; solvent B, 20% H_2O 80% acetonitrile, 0.04% formic acid).

MS analysis was run in data-dependent acquisition using a top five method (e.g., the five most intense ions with a positive charge between 2 and 4 analyzed during survey scan were selected for fragmentation during each scan cycle). Survey scans were performed in the Orbitrap at a resolution of 60,000 with a scan range of 450–650 *m/z* (450–750 *m/z* for absolute quantification). Peptides were fragmented using collision-induced dissociation (normalized collision energy, 35%; activation time, 30 ms; isolation width, 1.3 *m/z*) with resulting fragment ions (MS/MS scans) analyzed in the linear ion trap. Dynamic exclusion was enabled for all runs (maximal number of masses excluded at each time point [exclusion list size] 500; duration of exclusion for each mass: 40s).

Data analysis

Data analysis was performed with Proteome Discoverer 1.3. Peak lists were searched against Swissprot (date, November 2010; taxonomy filter, *Homo sapiens*) using Mascot software version 2.2.04 with no cleavage specificity selected. Precursor ion tolerance was set to 10 ppm and product ion tolerance to 0.6 Da. Filters used for postprocessing analysis included 5 ppm precursor ion tolerance, ionscore >20, and a maximum identification rank of 3. Peptides showing no HLA-A*02 motif were also excluded from further analysis. Percolator tool was used to evaluate peptide confidence based on *q*-value with a strict target false discovery rate (FDR) of 0.05 (high confidence) and a relaxed target FDR of 0.15 (medium confidence). Fragment spectra collected from peptides used for ranking were confirmed using synthetic peptides to ensure proper identification, in particular of

peptides showing low confidence (FDR < 0.15). Ordering of peptides according to their abundance was based on automatically calculated area under the curve (AUC) with a 2 ppm precursor ion window relying solely on identification rank 1 peptides. For absolute quantification, AUC of respective modified peptides was determined using Xcalibur Qual Browser with a mass accuracy of 2 ppm.

mRNA isolation and RT-PCR

Total RNA was isolated from 5×10^6 cells (EC, BLC, JY, T2, or LS174) using TRIzol reagent (Invitrogen Life Technologies) according to the manufacturer's protocol. After reverse transcription (SuperScript; Invitrogen Life Technologies), cDNA coding for the genes of interest was amplified using the following primers (all from Microsynth, Balgach, Switzerland): 5′-ATGGGGAAGGTGAAGGTCGG-3′ and 5′-AGGGATGATGTTCTGGAGAG-3′ for GAPDH, 5′-CTCTCCGCTCTCGCCCGCTA-3′ and 5′-AGGAATGGGGTGGGTGGCAG-3′ for PTRF, 5′-TGGACAATCACAATGGGAAT-3′ and 5′-CAAGAGCAAAGGAGGAAGC A-3′ for CD59, and 5′-CAAGAGGTGGAAACATACAG-3′ and 5′-AGCACCACCGTAGATACAG-3′ for DDX5. PCR for CD59 and GAPDH cDNA was performed with an initial denaturation step at 96°C for 5 min, then 35 cycles with 30 s denaturation at 96°C, 30 s annealing at 58°C, 1 min elongation at 72°C, followed by the final extension for 10 min at 72°C. Amplification of PTRF and DDX5 cDNA was done using annealing temperatures of 60 and 53°C, respectively.

Western blot

Cells (5×10^6; EC, BLC, JY, T2, or LS174) were solubilized in 200 μl lysis buffer (20 mM Tris HCl [pH 7.5], 150 mM NaCl, 1 mM DTT (Roche), 100 μM Na_3VO_4, 1 mM NaF, and 1% Triton-X in distilled H_2O containing the following protease inhibitors: 10 μg/ml aprotinin, 10 μg/ml leupeptin, 100 μM PMSF (all from Sigma-Aldrich), and 5 μg/ml pepstatin (AppliChem, Darmstadt, Germany). The protein concentration was measured by a Bio-Rad protein assay (Bio-Rad, Hercules, CA) according to the manufacturer's instructions. Per sample, 30 μg protein was loaded onto the gel. Nonreducing conditions were used for CD59; all other proteins were separated under reducing conditions. PTRF (43.5 kDa) and DDX5 (68 kDa) were separated on a 9% SDS-polyacrylamide gel, and CD59 (19 kDa) was separated on a 15% SDS-polyacrylamide gel using a Bio-Rad Mini-Protean 3 Cell. The proteins were transferred onto cellulose nitrate (Protran BA83; Whatman Schleicher & Schüll, Dassel, Germany) in TBS (25 mM Tris, 192 mM glycine, 20% methanol) and blocked in blocking solution (43.5 mM Tris-HCl, 6.5 mM Tris-base, 150 mM NaCl, 0.02% Tween 20) containing 5% fat-free milk. Proteins were detected with mAbs in saturating final concentrations specific for PTRF (1:250, P87520; BD Biosciences), DDX5 (1:750, Ab10261; Abcam, Cambridge, UK), and CD59 (1:200, MCA1054; Serotec, Oxford, U.K.). β-actin (1:80,000, AC-15; Sigma-Aldrich) was used as a loading control. All Ab incubation steps were performed for 60 min at room temperature in blocking solution. After washing three times 10 min with washing solution (43.5 mM Tris-HCl, 6.5 mM Tris-base, 150 mM NaCl, 0.02% Tween 20), the peroxidase-conjugated Abs (peroxidase-conjugated goat anti-mouse Ab [1:4000, 115-035-071; Jackson ImmunoResearch Laboratories, Westgrove, PA], peroxidase-conjugated rabbit anti-goat Ab [1:2000, P0449; Dako, Glostrup, Denmark]) were added and detected by the chemiluminescence substrate (SuperSignal West Pico; Pierce Chemical/Perbio Science, Helsingborg, Sweden). Emitted light was collected on a photographic film (Biomax; Eastman Kodak, Rochester, NY) and automatically developed (Curix 60; Agfa-Gevaert, Mortsel, Belgium).

Peptides synthesis

The HLA-A*02–restricted peptides GILGFVFTL ($Flu_{58–66}$), FIDSYICQV ($SMCY_{311–319}$), SLLDKIIGA ($PTRF_{56–64}$), SLSEKTVLL ($CD59_{106–114}$), and YLLPAIVHI ($DDX5_{168–176}$) were synthesized on an automated peptide synthesizer 433A (Applied Biosystems, Foster City, CA) using standard 9-fluorenylmethyloxycarbonyl/*tert*-butyl strategy. Peptides used for absolute quantification were further purified using preparative HPLC to ensure purities >95%.

Competitive binding assay

Peptide affinity for HLA-A*02 was quantified by a competitive binding assay. T2 cells were loaded with 10^{-8} M $Flu_{58–66}$ in the simultaneous presence of different concentrations of either $SMCY_{311–319}$, $DDX5_{168–176}$, $PTRF_{56–64}$, or $CD59_{106–114}$. The competing peptides were present at increasing concentrations ranging from 10^{-12} M to 10^{-4} M. Cells were incubated overnight at 37°C in complete RPMI 1640 (2.5% FCS). The next day, a calcein-release cytotoxicity assay was performed as described previously using a CTL line specific for $Flu_{58–66}$-HLA-A*02 (13). In the simultaneous presence of 10^{-8} M $Flu_{58–66}$ and increasingly higher concentrations of the peptide under investigation, target cell killing by $Flu_{58–66}$-specific CTL was incrementally inhibited.

T2 peptide binding assay

TAP-deficient T2 cells express largely unloaded MHC class I molecules that are unstable, and therefore HLA-A*02 surface levels are low. When T2 cells were incubated overnight at 37°C with 1×10^{-5} M peptide in complete RPMI 1640 (supplemented with only 2.5 instead of 10% FCS), HLA-A*02 surface levels did increase due to stabilization by specific peptide binding. Cells were washed and surface expression of HLA-A*02 was determined by FACS using BB7.2 mAb (1:100; gift from Peter Cresswell, Yale Medical School, New Haven CT) or an isotype control Ab (1:100; product no. mca928; Serotec), respectively. After washing, cells were incubated with FITC-labeled goat anti-mouse IgG secondary Ab (1:200, product no. 115-095-003; Jackson ImmunoResearch Laboratories). All incubations were strictly performed on ice. Data acquisition was performed on a Cyan ADP FACS instrument using Summit Software (Dako). Additionally, 1×10^5 cells per sample were collected in the lymphocyte gate and analyzed. The change in mean fluorescence intensity (ΔMFI) was calculated by subtracting the MFI with isotype control Ab from the MFI with BB7.2 mAb. The fluorescence index was calculated as ΔMFI with peptide/ΔMFI without peptide (17).

Decay and half-life of the peptide/MHC class I complex

For the determination of half-life of the HLA-A*02/peptide complexes, T2 cells were loaded with peptide overnight at 37°C in complete RPMI 1640 (2.5% FCS). Peptide concentrations used for pulsing were adjusted to equalize the fluorescence index of T2 cells. After washing extensively, cells were again incubated at 37°C. At indicated time points (0, 2, 6, 24 h) aliquots were stained for HLA-A*02 as described above and staining intensity assessed by specific ΔMFI was determined by flow cytometry (17).

Target cell treatment by small interfering RNA

The following small interfering RNAs (40 μM stock concentrations) from Microsynth were used to knock down precursor proteins of prevalent peptides: CD59 small interfering RNA (siRNA), 5′-GAAGUCUAAGAGUGAAGUATT-3′; PTRF siRNA, 5′-GAGGAAAGAUUGAAUCCUAT-3′; DDX5 siRNA, 5′-GCAGAUAGAAUGCUUGAUATT-3′, and mock siRNA, 5′-AGGUAGUGUAAUCGCCUUGTT-3′. Early passage EC or exponentially growing JY cells were seeded 1 d before transfection at 10^5 cells per well in complete medium 199 without penicillin/streptomycin in a gelatin-coated six-well plate. The next day, the medium was replaced with 2.5 ml prewarmed complete medium 199 without penicillin/streptomycin. RNA complexes were prepared as follows (volumes for one well of a six-well plate): 10 nM siRNA was slowly mixed with Lipofectamine RNAiMAX transfection reagent (Invitrogen) in Opti-MEM I GlutaMAX medium (Life Technologies) according to the manufacturers' instructions. Then, the complexes were added dropwise and gently to the cells. The medium was changed after 16 h and the cells incubated at 37°C for indicated times. Cells were either analyzed by Western blot for detecting protein levels or used for cytotoxicity assays (13) or flow cytometry.

Calcein-release–based cytotoxicity assay

CTL-mediated target cell lysis was measured by a calcein-release assay as described (13). Adherent EC grown to confluency in 96-well flat-bottom plates (Falcon) or JY cells were loaded for 30 min at 37°C with 20 μM calcein-AM (Molecular Probes/ Invitrogen) in serum-free medium 199. Cells were then washed and bleached for 2 h at 37°C in complete medium 199. After washing twice with complete medium 199 and then assay medium (medium 199, 2% FCS, 5 mM HEPES, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin), 50 μl assay medium was added to 10,000 target cells per well. CTL were washed, counted, and added to the calcein-loaded target cells at an E:T ratio of 10:1 (final volume, 200 μl/well) and spun down. Spontaneous release was determined by adding assay medium to the target cells; maximum release was obtained by adding lysis buffer (50 mM sodium borate in 0.1% Triton X-100 [pH 9.0; both from Sigma-Aldrich]). After incubation for 2 h at 37°C, 75 μl supernatant was carefully removed and transferred to another 96-well, flat-bottom plate. Released calcein was measured in a fluorescence multiwell plate reader (SPECTRAmax GEMINI-XS; Molecular Devices; excitation wavelength, 485 nm; emission wavelength, 538 nm). Percentage specific lysis was calculated as [(sample release − spontaneous release)/(maximum release − spontaneous release)] × 100%.

Statistical methods

Mean values between groups were compared using the Student *t* test. Unless indicated otherwise, means ± SD of triplicates are shown.

Results

*HLA-A*02–restricted peptides presented by EC*

We characterized the endothelial and leukocyte-derived HLA-A*02–restricted peptide profile in three independent experiments analyzing cells from three healthy HLA-A*02–positive males. EC and BLC were grown side-by-side under virtually identical conditions and harvested using the same procedure to avoid methodological bias. HLA surface expression was considerably higher in BLC compared with EC from all three donors, as determined by flow cytometry using either HLA-A*02–specific mAb BB7.2 or pan-HLA class I-specific mAb W6/32 (Fig. 1A). As expressed in median values and interquartile range (shown in parentheses), EC lines had on average 22 (16–29) $\times$ 10^3 HLA-A*02 molecules or 88 (86–137) $\times$ 10^3 HLA class I molecules per cell. Corresponding BLC expressed on average 142 (129–151) $\times$ 10^3 HLA-A*02 or 482 (436–515) $\times$ 10^3 HLA class I molecules per cell. Therefore, the total number of HLA ligands was expected to be at least 5-fold lower in EC compared with BLC.

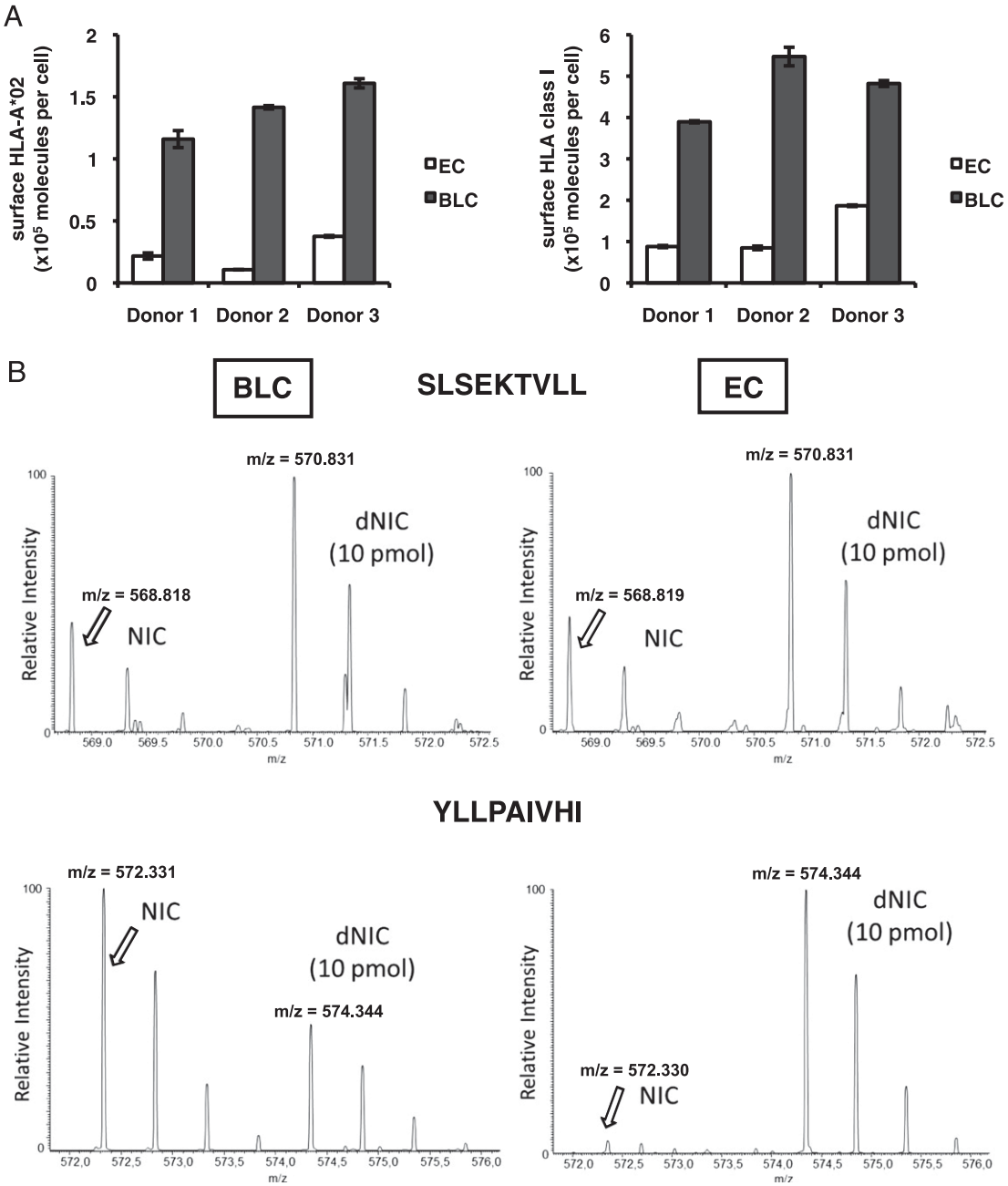

FIGURE 1. (**A**) Quantification of surface HLA-A*02 molecules and total HLA class I molecules on EC and BLC. HLA-A*02 molecules represent ~25% of all HLA class I molecules, both on EC and on BLC from positive donors. EC have ~6-fold less HLA-A*02 or HLA class I molecules, respectively, than do BLC. (**B**) Quantification of HLA-A*02 ligands SLSEKTVLL (*upper panel*) and YLLPAIVHI (*lower panel*) from BLC (*left*) and EC (*right*). Synthetic peptides labeled with deuterated nicotinic acid (dNIC) were used for calibration (arrow). Ten picomoles of each spiked peptide was injected together with nicotinylated (NIC) peptide mixtures derived from HLA-A*02 ligand isolation and analyzed by LC-MS. The nicotinylated native peptide is marked by an arrow. Absolute quantification of SLSEKTVLL, the most prevailing ligand found on ECs, reveals comparable quantities (~3 pmol) presented on EC as well as on BLC. Allotypic HLA-A*02 ligand YLLPAIVHI, which is present on EC at negligible concentrations (arrow in the *lower right panel*), confirms the relative predominance of SLSEKTVLL on EC. Mass spectra show the relative intensity averaged over the retention time of respective precursor ion peaks within the ion chromatogram. Shown is the result obtained from donor 1. Graphics were generated with Xcalibur 2.07 QualBrowser.

The entire pool of peptides bound to endothelial or leukocyte HLA-A*02 molecules was isolated, fractionated, and the individual components were characterized by tandem mass spectrometry. As expected by the use of the A*02-specific Ab BB7.2, most peptides encompassed the allele-specific peptide motif of HLA-A*02 characterized by aliphatic anchor amino acids in position 2 and at the C terminus. A total of 56 HLA-A*02 ligands were identified from three EC lines whereas ˃1400 HLA-A*02 ligands were characterized in BLC extraction experiments (Supplemental Tables I, II). Note that $SMCY_{311–319}$, a male-specific, immunodominant minor histocompatibility Ag (18), was never identified in these isolates, neither in EC nor in BLC. The amino acid sequences of the isolated HLA-A*02–restricted peptides were used for sequence homology searches, and the source proteins from which these peptides derive were identified. A comprehensive listing of HLA-A*02 ligands, their source proteins, and MS-relevant parameters are given in Supplemental Tables I and II. EC and BLC shared 25 of the 56 identified peptide species, among them processing products of RNA helicase DDX5 (YLLPAIVHI) and coatomer subunit gamma, COPG (AIVDKVPSV); both peptides have been repeatedly found in HLA-A*02–extracted peptide pools obtained from human tissues. Moreover, YLLPAIVHI has been classified as an allotypic peptide presented on most HLA-A*02 expressing cells and characteristic for this HLA allotype (19). Thirty-five peptides were found exclusively on EC but not on autologous BLC.

Two peptides, SLLDKIIGA and SLSEKTVLL, yielded extraordinarily high signals in EC compared with other cells and were consistently identified in all three EC donors analyzed in this study, suggesting that these peptides are constitutively processed and presented by the MHC class I pathway of EC (Table I). The proteins from which these two peptides arise are polymerase I and transcript release factor (PTRF) (20, 21) and the complement inhibitor CD59 (22). Both HLA-A*02–restricted peptides have been isolated previously from other tissues. However, the relative amounts of PTRF- and CD59-derived peptides appeared higher in EC compared with any other cell line and tissue from which these peptides have been previously isolated. Comparing the AUC values of the different peptides in LC-MS experiments, SLSEKTVLL was detected with highest abundance in EC lines closely followed by SLLDKIIGA, whereas both peptides were not among the top rankings with respect to AUC values in BLC (Table I).

To achieve an exact quantitative determination of peptide amounts, stable isotope labeling experiments were performed. Natural HLA-A*02 ligands from either source were labeled with nicotinic acid, whereas synthetic calibrating peptides were labeled with deuterated nicotinic acid (16). Spiking experiments using defined amounts of calibrating peptides led to a precise quantification of HLA-A*02 ligands (Fig. 1B, Supplemental Table III) and confirmed the estimations from AUC values. Both SLSEKTVLL (CD59) and SLLDKIIGA (PTRF) represented by far the most abundant peptides of EC, dominating the HLA-A*02–mediated peptide repertoire (Table II). Although also occurring in HLA-A*02 ligand pools of BLC, they play a minor quantitative role there and are concealed by vast copy numbers of other HLA-A*02 ligands such as YLLPAIVHI (DDX5) and AIVDKVPSV (COPG) (Table II). In particular, the PTRF-derived peptide SLLDKIIGA was hardly detectable on BLC (Table I), highlighting the observation that in no other tissues or cell lines analyzed so far, SLLDKIIGA and SLSEKTVLL were as dominant as in EC. These two peptides were repetitively identified as the most prevalent HLA-A*02 ligands in these cells. Knowing the total EC end BLC number per donor that entered these experiments, and knowing the number of HLA-A*02 molecules per cell, we were able to calculate that SLSEKTVLL and SLLDKIIGA together represent >50% of all HLA-A*02–restricted peptides per single EC but <5% of the peptides presented by BLC.

*The molecular basis for abundant and endothelial cell-selective presentation of HLA-A*02–bound peptides*

The most obvious explanation and logical prerequisite for the preferential presentation of MHC class I bound $PTRF_{56–64}$ and $CD59_{106–114}$ on EC would be the strong and cell type-specific expression of PTRF and CD59 genes. Therefore, we compared the mRNA and protein levels of DDX5, PTRF, and CD59 in EC and syngeneic EBV-immortalized BLC from the same individual as the EC, in the HLA-A*02–positive BLC line JY, and in TAP-deficient T2 cells and LS174 colon cancer cells (Fig. 2). PTRF

Table I. Peptides identified in EC (*top*) and BLC (*bottom*) together with their ranking based on the relative abundance estimated by the AUC of respective precursor ions

	EC Donor 1		EC Donor 2		EC Donor 3	
Sequence	AUC	Rank	AUC	Rank	AUC	Rank
SLLDKIIGA	1.57×10^7	2	3.53×10^7	3	4.82×10^7	3
SLSEKTVLL	5.48×10^7	1	9.46×10^7	1	9.29×10^7	1
YLLPAIVHI	ND		ND		ND	
AIVDKVPSV	ND		4.43×10^6	5	4.19×10^6	9
ILMEHIHKL	ND		ND		4.00×10^5	26
VLIPKLPQL	ND		ND		ND	
YLPEDFIRV	ND		ND		ND	
FIDSYICQV	ND		ND		ND	
ILDQKINEV	3.48×10^6	6	3.66×10^6	9	5.60×10^6	8
	BLC Donor 1		BLC Donor 2		BLC Donor 3	
	AUC	Rank	AUC	Rank	AUC	Rank
SLLDKIIGA	ND		ND		ND	
SLSEKTVLL	2.87×10^7	226	6.95×10^6	80	ND	
YLLPAIVHI	2.25×10^8	37	ND		ND	
AIVDKVPSV	1.30×10^9	3	1.98×10^8	2	1.54×10^5	55
ILMEHIHKL	2.43×10^8	31	5.65×10^6	108	ND	
VLIPKLPQL	2.83×10^9	1	7.72×10^5	400	ND	
YLPEDFIRV	2.88×10^8	24	9.50×10^6	56	ND	
FIDSYICQV	ND		ND		ND	
ILDQKINEV	6.99×10^8	6	1.56×10^8	4	ND	

Table II. Absolute quantification of selected HLA ligands in two consecutive runs, each containing 20% of complete isolation per cell line of EC (~10^8 cells) and BLC (~4×10^8 cells) from donor 1 with 0.3 (run 1) or 10 pmol (run 2) of spiked calibrating peptides

Sequence	Run 1	Run 2
EC donor 1		
SLSEKTVLL	3396.37	2864.74
SLLDKIIGA	355.31	836.80
AIVDKVPSV	347.41	114.09
ILDQKINEV	335.29	121.39
YLLPAIVHI	11.15	62.07
BLC donor 1		
AIVDKVPSV	369,079.91	128,173.75
YLLPAIVHI	54,923.29	23,609.07
ILDQKINEV	209,735.02	23,182.60
SLSEKTVLL	10,387.56	2764.77
SLLDKIIGA	30.47	157.82

was expressed both at the RNA and protein levels exclusively in EC and was not found in any other cell line analyzed (Fig. 2). CD59 was expressed at the RNA level in all cell lines analyzed (Fig. 2A). At the protein level, CD59 was expressed at highest level in EC and faintly in the two BLC lines analyzed, but not in T2 or LS174 colon cancer cells (Fig. 2B). DDX5, the ubiquitously expressed protein that is the source of the allotypic reference peptide YLLPAIVHI ($DDX5_{168–176}$), which is present at large quantities in the peptide binding groove of HLA-A*02 molecules of most tissues (19), was found in all cell lines both at the RNA as at the protein level in similar amounts (Fig. 2). Interestingly, the

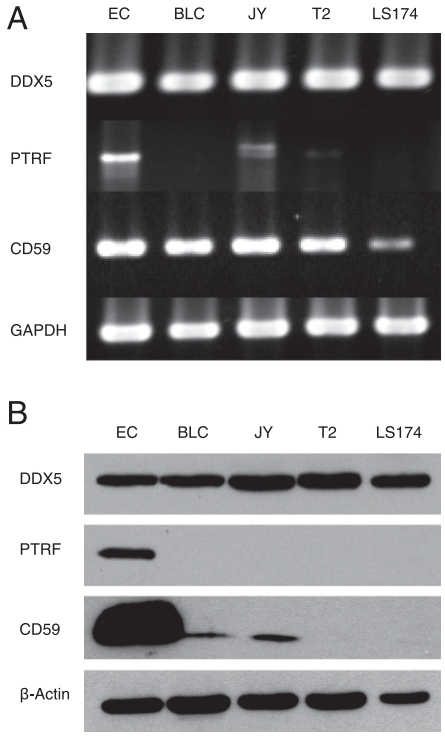

FIGURE 2. mRNA expression and cellular representation of source proteins that contain the HLA-A*02 presented endothelial peptide sequences. (**A**) Total RNA for RT-PCR was obtained from cultured HUVEC (EC), BLC, the B cell line JY, T2 cells, and the colon carcinoma cell line LS174. Shown are the RT-PCR products of PTRF, CD59, DDX5, and GAPDH transcripts. (**B**) Western blot was performed with whole cell lysates from EC, BLC, JY, T2 cells, and LS174. All cells were analyzed for the presence of the proteins PTRF, CD59, and DDX5. β-actin was used as a loading control.

abundant structure protein β-actin was equally expressed in all cell lines at the protein level (Fig. 2B). According to the SYFPEITHI algorithm (http://www.syfpeithi.de) (23), β-actin contains several peptide motifs that match criteria for HLA-A*02 binding. However, none of these putative HLA-A*02–restricted β-actin peptides was ever detected in the cell isolates analyzed, neither in EC nor in BLC (Supplemental Tables I, II).

*Peptide affinity for HLA-A*02*

High peptide affinity for HLA-A*02 may further contribute to the dominant representation of certain peptides in EC-derived profiles. We compared the endothelial peptides $PTRF_{56–64}$ and $CD59_{106–114}$, as well as the ubiquitous $DDX5_{168–176}$ and $SMCY_{311–319}$, for binding to HLA-A*02. A functional competition assay was performed to measure possible differences in peptide affinity for HLA-A*02. In this assay, the peptide concentration was determined at which target cell killing by $Flu_{58–66}$-specific CTL in the presence of 10^{-8} M $Flu_{58–66}$ peptide was inhibited by 50% (Fig. 3A, 3B, Table III). $SMCY_{311–319}$ required $0.3 \pm 0.2 \times 10^{-5}$ M peptide concentration to reduce $Flu_{58–66}$-specific killing by 50%. For $DDX5_{168–176}$ $0.8 \pm 0.4 \times 10^{-5}$ M, for $PTRF_{56–64}$ $1.5 \pm 0.7 \times 10^{-5}$ M, and for $CD59_{106–114}$ $2.1 \pm 1.1 \times 10^{-5}$ M peptide was necessary to inhibit $Flu_{58–66}$-specific lysis by 50%. This indicates that peptide affinity for HLA-A*02 is lowest for the two most abundant endothelial peptides $PTRF_{56–64}$ and $CD59_{106–114}$. This unexpected result was endorsed by a second independent experimental approach, that is, a T2 binding assay (24). Surface expression of HLA-A*02 was determined by flow cytometry after overnight pulsing of T2 cells by 10^{-5} M peptide (Fig. 3C). $SMCY_{311–319}$ and $DDX5_{168–176}$ were the strongest binders, increasing the fluorescence index 3.2 ± 0.2- and 2.9 ± 0.1-fold, respectively (Fig. 3C, Table III). $PTRF_{56–64}$ and $CD59_{106–114}$ showed significantly weaker binding, increasing HLA-A*02 expression 2.2 ± 0.1- and 1.4 ± 0.1-fold, respectively ($p = 0.002$ and $p = 0.003$, respectively). These results confirmed the observations made in the competitive binding assay.

Peptide concentrations could be adjusted according to the findings of the competitive $Flu_{58–66}$-binding assay to similar surface levels of HLA-A*02 in T2 cells (Fig. 3D). Compared with $SMCY_{311–319}$, a 2.4-fold higher concentration for $DDX5_{168–176}$, a 4.4-fold higher concentration for $PTRF_{56–64}$, and an 8-fold higher peptide concentration for $CD59_{106–114}$ was necessary to compensate for the weaker binding of the respective peptides (Fig. 3D).

Stability of the peptide/MHC class I complex

Finally, the decay of the peptide/HLA-A*02 complexes could make a difference in surface peptide presentation of cells. Half-lives of peptide/HLA-A*02 complexes were determined by measuring the HLA-A*02 surface expression on T2 cells at indicated time points after pulsing with adjusted peptide concentrations (Fig. 4). T2 cells were loaded with the peptide of interest for 24 h, unbound peptide was washed away, and HLA-A*02 surface expression was measured by flow cytometry at different time points (0, 2, 6, 24 h). $SMCY_{311–319}$, $DDX5_{168–176}$, and $PTRF_{56–64}$ showed similar kinetics with half-lives of 6.0, 5.8, and 7 h, respectively (Fig. 4). However, the half-life of $CD59_{106–114}$ was only 2.8 h and therefore substantially shorter compared with the other peptides. Stability of HLA-A*02/peptide complexes on the cell surface does not contribute to the dominant presentation of $CD59_{106–114}$.

Modulation of endothelial CTL susceptibility by source protein expression

The dominant presentation of certain cell-specific peptides on EC could also compete with the surface expression of immunodominant CTL epitopes, for example, the HLA-A*02–restricted

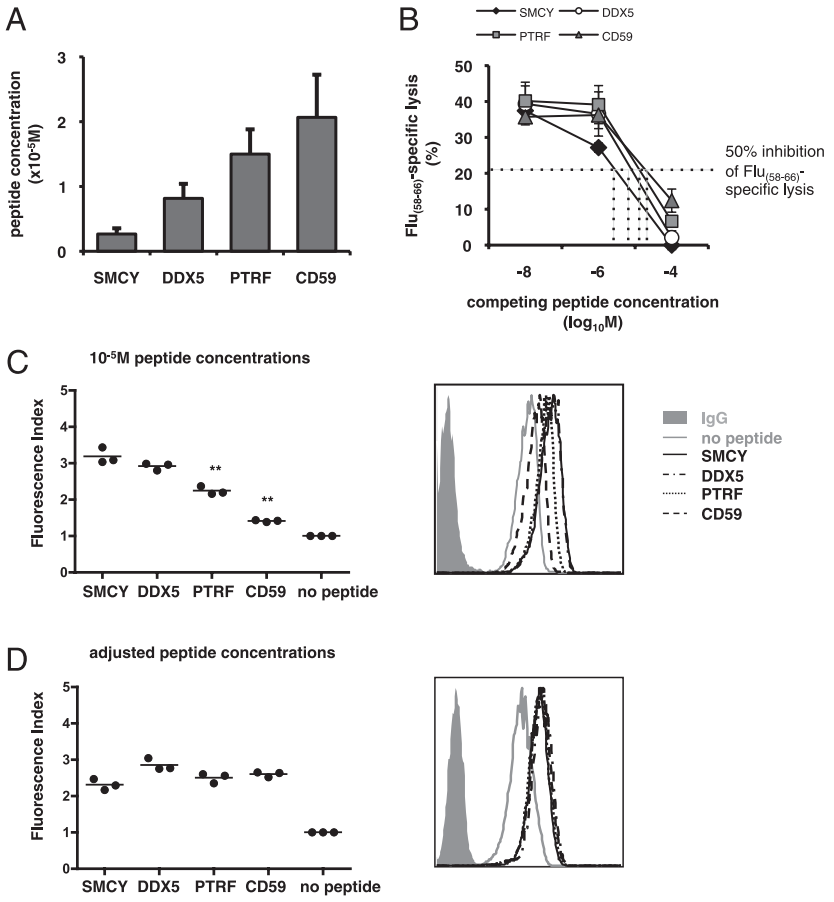

FIGURE 3. Peptide affinity for HLA-A*02. Competitive binding and T2 peptide binding assay were performed for four different allo- and auto-peptides ($SMCY_{311–319}$, $DDX5_{168–176}$, $PTRF_{56–64}$ and $CD59_{106–114}$). (**A**) The competitive binding assay shows the peptide concentration (10^{-5} M) in the presence of 10^{-8} M $Flu_{58–66}$, at which target killing by $Flu_{58–66}$-specific CTL was inhibited by 50%. (**B**) Specific lysis of T2 cells loaded with 10^{-8} M $Flu_{58–66}$ and different concentrations of the peptide of interest ($SMCY_{311–319}$, $DDX5_{168–176}$, $PTRF_{56–64}$, and $CD59_{106–114}$) ranging from 10^{-4} to 10^{-8} M by $Flu_{58–66}$-specific CTL. The dashed line indicates the peptide concentration at which target cell killing by $Flu_{58–66}$-specific CTL was inhibited by 50%. (**C** and **D**) For the T2 binding assay, HLA-A*02 positivity of peptide-loaded T2 cells was analyzed by flow cytometry. The fluorescence index was calculated as ΔMFI with peptide/ΔMFI without peptide. (C) Fluorescence index and typical histograms with 10^{-5} M peptide concentrations are shown, and (D) with peptide concentrations adjusted to binding differences calculated from the competitive binding assay. For each peptide three independent competition and binding assays were performed. **Significant changes compared with fluorescence index for SMCY, $p < 0.05$.

minor histocompatibility Ag $SMCY_{311–319}$. By this mechanism EC could be protected at least partially from CTL-mediated lysis (13). To test this speculative hypothesis, precursor proteins of the abundant EC peptides were transiently eliminated by RNA interference and EC were then used as targets in a CTL assay. siRNA designed for DDX5, PTRF, and CD59 using a publically available algorithm (www.microsynth.ch) (25) were used in EC and BLC and protein levels were assessed by Western blot (Fig. 5A). In EC, DDX5 knockdown was complete after 24 h and lasted for ˃96 h. CD59 protein levels started to decrease at 48 h and were undetectable by this method after 72 h. PTRF elimination also started after 48 h. The protein reached minimal levels (20%) by 72 h and started to increase after 96 h. Based on these protein kinetics, cells were used after 72 h as targets in cytotoxicity assays. Simultaneous knockdown of $PTRF_{56–64}$, $CD59_{106–114}$, and $DDX5_{168–176}$ on male EC should render these cells more susceptible for killing by $SMCY_{311–319}$-specific CTL. We found that after treatment of EC with combined siRNAs for 72 h, specific killing raised from 14 ± 5 to 21 ± 6% ($p = 0.002$; Fig. 5B). Killing of the male B cell line JY treated with either specific or mock siRNA remained unchanged (Fig. 5C). The seemingly small 7% difference in specific killing translates into a 90-fold higher amount of peptide expressed by EC (0.9×10^2 M) when the cytolytic activity on treated and untreated EC is compared with the peptide titration curve using $SMCY_{311–319}$-pulsed T2 cells as targets (Fig. 5D). HLA-A*02 expression levels of target cells were not changed by siRNA treatment after up to 72 h (Fig. 5E).

Discussion

In this study, we analyzed the endothelial peptide repertoire presented by HLA-A*02 and compared it with the repertoire presented by syngeneic B lymphoblastoid cells. We demonstrate that EC present a quantitatively different peptide repertoire that may contribute to the protection of EC from CTL-mediated lysis by competition with immunogenic peptides for the MHC class I binding sites.

Compared to leukocyte-derived cells, the peptide repertoire presented by EC showed a consistent prevalence of two peptides,

Table III. Binding affinity and half-life of various peptide/HLA-A*02 complexes

	Competitive Binding Assay (Peptide Concentration for 50% Inhibition, M)	T2 Binding Assay (Fold Increase of Fluorescence Index)	Half-Life Assessment (h)
$Flu_{58–66}$	Not applicable	Not applicable	Not applicable
$SMCY_{311–319}$	$0.3 \pm 0.2 \times 10^{-5}$	3.2 ± 0.2	6.0
$DDX5_{168–176}$	$0.8 \pm 0.4 \times 10^{-5}$	2.9 ± 0.1	5.8
$PTRF_{56–64}$	$1.5 \pm 0.7 \times 10^{-5}$**	2.2 ± 0.1**	7.0
$CD59_{106–114}$	$2.1 \pm 1.1 \times 10^{-5}$**	1.4 ± 0.1**	2.8

See also Fig. 3.
**$p < 0.05$, significantly different from $SMCY_{311–319}$.

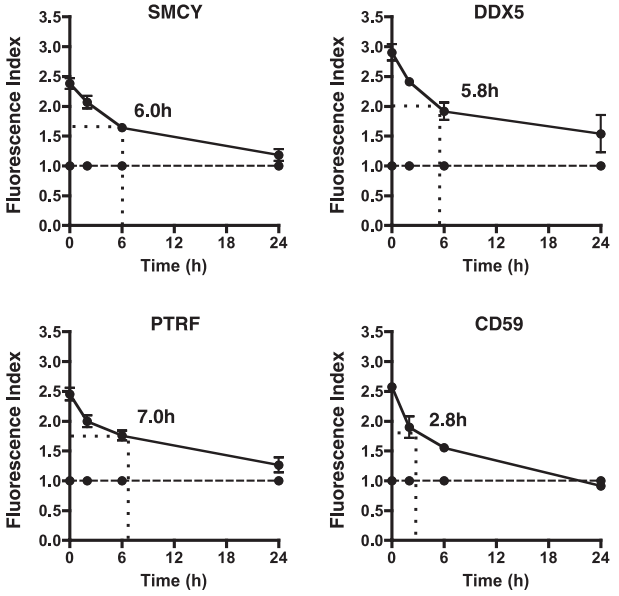

FIGURE 4. Half-life of HLA-A*02–bound peptides. T2 cells were loaded with the peptides ($SMCY_{311–319}$, $DDX5_{168–176}$, $PTRF_{56–64}$, and $CD59_{106–114}$) in adjusted concentrations according to the competitive binding assay and HLA-A*02 surface positivity was analyzed by flow cytometry at indicated time points (0, 2, 6, 24 h). For each peptide three independent experiments were performed.

$PTRF_{56–64}$ and $CD59_{106–114}$. We found that the predominance of these two endothelial peptides was caused by the abundant expression of the source proteins PTRF and CD59. PTRF has been identified as a major caveolae-associated protein (18), and caveolae are particularly abundant in endothelial cells and adipocytes. BLC and EC both express CD59 protein, although the amount is substantially higher in EC. This may explain the vast abundance of $CD59_{106–114}$ peptide on EC compared with BLC. Note that $SMCY_{311–319}$, an immunodominant HLA-A*02–bound peptide originally described as minor histocompatibility Ag (19), was never identified in the isolates from male cells, neither in BLC nor in EC. Similarly, we could never find peptides derived from β-actin in these isolates, although it is a strongly expressed structure protein, contains several possible HLA-A*02 binding motifs, and although YALPHAILRL and ALAPSTMKI (both from β-actin) were identified previously on tumor tissue.

We assume that for SMCY and particularly for β-actin, rather low protein synthesis or degradation rates are involved as mechanisms for quantitatively low surface expression of these peptides on both EC and BLC (26, 27). Male BLC (but not male EC) are recognized and killed by CTL specific for $SMCY_{311–319}$ (13). This confirms that the peptide isolation assay is substantially less sensitive than the calcein-release–based killing assay for the surface detection of peptide motifs. It cannot be excluded that cysteine containing peptide $SMCY_{311–319}$ is more prone to chemical modifications during the isolation process, which can lead to a decreased intensity of the unmodified peptide and this may interfere with peak annotation after analysis. However, even under reducing conditions and subsequent modification of cysteinyl residues with iodoacetamide, SMCY could not be detected in any of the runs (data not shown).

We further found that the strong expression and putatively also turnover of the precursor proteins of $PTRF_{56–64}$ and $CD59_{106–114}$ are able to compensate for the low binding affinity of the endothelial peptides to HLA-A*02 and their short half-life. It has been shown that one important factor for the immunogenicity of a peptide is the stability of the peptide/MHC complex (28, 29).

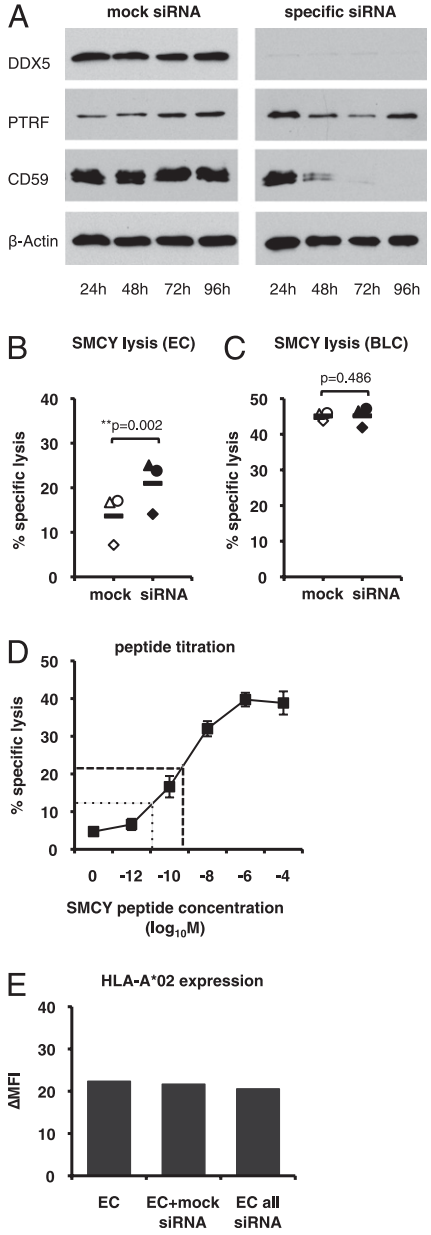

FIGURE 5. Knockdown of abundant endothelial peptides. (**A**) Western blot showing protein levels of DDX5, PTRF, and CD59 in EC after treatment with either mock siRNA or specific siRNA at different time points after knockdown (24, 48, 72, 96 h). β-actin is shown confirming similar protein loads. (**B** and **C**) Specific lysis of male EC (B) or JY cells (C) treated with mock or specific siRNA by $SMCY_{311–319}$-specific CTL. Shown are the results of three independent experiments. (**D**) Peptide titration of $SMCY_{311–319}$-loaded T2 cells. The dotted line indicates the specific lysis of EC treated with mock siRNA, the dashed line of EC treated with specific siRNA. (**E**) HLA-A*02 levels on EC after treatment with either mock or specific siRNA.

Peptides with lower affinity for the MHC class I molecule may form less stable peptide/MHC complexes, leading to decreased immunogenicity. The presentation of particularly weak binding peptides may therefore be another mechanism to protect EC from CTL-mediated lysis.

The immunological significance of cell type-specific peptide presentation has been reported previously in the context of autoimmune diseases. Cell type-specific Ag expression may play an important role in organ- or cell-specific T cell-mediated autoimmune diseases such as type 1 diabetes mellitus or multiple sclerosis.

In a murine model of type 1 diabetes, the insulin-producing β cells of the pancreas are selectively targeted by autoreactive $CD8^+$ T cells (30). In murine multiple sclerosis, autoreactive $CD8^+$ T cells responsive to myelin-derived peptides have been reported that have the potential to kill selectively MHC class I-matched oligodendrocytes (31–33). In the context of allogeneic bone marrow transplantation it has been shown that the restricted expression of minor histocompatibility Ags on hematopoietic cells, including leukemic cells, can separate the beneficial graft-versus-leukemia from the harmful graft-versus-host effect of ubiquitously expressed minor histocompatibility Ags (34, 35). However, for endothelial cells, the cell type selective peptide repertoire seems to have an opposite effect. We provide evidence that the predominant presentation of the endothelium-specific Ags $PTRF_{56–64}$ and $CD59_{106–114}$ can protect EC from CTL-mediated lysis by competing with the immunodominant peptide $SMCY_{311–319}$ for the binding site on HLA-A*02 molecules. Knockdown of the abundant endothelial source proteins PTRF, CD59, and DDX5 by RNA interference led to an increased presentation of endogenously processed male-specific peptide $SMCY_{311–319}$, proving that without the abundant endothelial peptides EC become better targets for $SMCY_{311–319}$-specific CTL. Importantly, this mechanism does not restore EC as CTL targets to the same level as BLC. Lack of activating costimulators (36) or production of inhibitory cytokines (37, 38) might further contribute to the immune privilege of vascular endothelium. However, it has been shown in previous work that different cells process and present the same peptide source proteins in quantitatively different ways (39). The immediate consequences were differences in target cell recognition of these two cell types by the same CTL. Our observations confirm these findings and contribute an additional, novel aspect of quantitative peptide abundance: competitive inhibition of immunodominant epitope presentation. We do not know whether this surface peptide modulation is significant in vivo, that is, whether it protects cells or tissues from immune-mediated injury. However, Bolinger et al. (40) showed in a mouse model that vascular EC expressing minor histocompatibility Ags are immunologically ignored by Ag specific $CD8^+$ T cells. This phenomenon could be explained by low surface Ag expression levels on EC. Further limitations of our work is the restraint of repertoire analysis to one HLA-A species, HLA-A*02. This may preclude generalization of our findings to the MHC class I repertoire of cells or tissues.

In summary, we show that in vitro, human EC present a quantitatively different HLA-A*02–restricted peptide repertoire compared with syngeneic BLC and by this feature are at least partially protected from CTL-mediated lysis. The presented peptide profile can be modulated by knocking down the source proteins in target cells. The interference with source protein expression may be a novel approach to modify target cell properties.

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Disclosures

The authors have no conflicts of interest.

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