Mass spectrometry-assisted identification of ADAMTS13-derived peptides presented on HLA-DR and HLA-DQ

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Supplemental information

Isolation of PBMCs and generation of immature dendritic cells

Peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats obtained from healthy donors. Blood was drawn in agreement with Dutch regulations and after approval from the Sanquin Ethical Advisory Board in accordance with the declaration of Helsinki. Briefly, a Ficoll Paque density gradient (GE healthcare, Eindhoven, The Netherlands) was used to separate the PBMCs from the buffy coat. The ring fraction containing the PBMCs was resuspended in the PBS diluted plasma fraction and monocytes were enriched by elutriation (Avantie J-26 XPI, Beckman Coulter, Woerden, The Netherlands). The monocytes were subsequently differentiated into immature mo-DCs by culturing them in Cellgro medium (CellGenix, Freiburg, Germany) supplemented with 800 U/ml IL-4 and 1000 U/ml GM-CSF (both from CellGenix, Freiburg, Germany) for 5 days.

HLA-DR and HLA-DQ genotyping

Genotyping for HLA-DRB1 and HLA-DQB1 was performed in house using PCR-Sanger Sequencing Based Typing (PCR-SBT) amplification primers of the SBT*excellerator*® HLA-DRB1/DQB1 kit (Genome Products, Utrecht, The Netherlands). HLA-DQA1 typing was performed by GenDX (Utrecht, The Netherlands) using a next generation sequencing workflow (NGSgo®, Genome Products, Utrecht, The Netherlands).

Mass spectrometry analysis of purified peptides

Peptides were separated by nanoscale C18 reverse phase chromatography coupled on line to an Orbitrap Fusion Tribrid mass spectrometer (Thermo Scientific) via a nanoelectrospray ion source (Nanospray Flex Ion Source, Thermo Scientific). Peptides were loaded on a 20 cm 75–360 µm inner-outer diameter fused silica emitter (New Objective) packed in-house with ReproSil-Pur C18-AQ, 1.9 µm resin (Dr Maisch GmbH). The column was installed on a Dionex Ultimate3000 RSLC nanoSystem (Thermo Scientific) using a MicroTee union formatted for 360 µm outer diameter columns (IDEX) and a liquid junction. The spray voltage was set to 2.15 kV. Buffer A was composed of 0.5% acetic acid in water and buffer B of 0.5% acetic acid, 19.5% water, 80% acetonitrile. Peptides were loaded at 300 nl/min at 5% buffer B, equilibrated for 22 minutes at 5% buffer B (0-22 min) and eluted by increasing buffer B from 5-40% (22-62 min), followed by a 10 minute wash to 90%, 3 minute hold at 90%, a 2 minute ramp back to 5% and a 5 min regeneration at 5%. Survey scans of peptide precursors from 400 to 1500 m/z were performed in the Orbitrap Fusion Tribrid mass spectrometer at 120K resolution (at 200 m/z) with a 1.5 x 10^5 ion count target. Tandem mass spectrometry of the 5 most intense precursors was performed by isolation with the quadrupole with isolation width 1.6, higher-energy collisional dissociation (HCD) fragmentation with normalized collision energy of 30, and rapid scan mass spectrometry analysis in the ion trap. The dynamic exclusion duration was set to 60 s with a 10 ppm tolerance around the selected precursor and its isotopes. Mono-isotopic precursor selection was turned on. The MS2 ion count target was set to 500 and the

max injection time was 35 ms. Only those precursors with charge state 2 and up were sampled for MS2. All data were acquired with Xcalibur software.

Evaluation of ADAMTS13 peptide immunogenicity using in silico tools

In silico approach developed by De Groot *et al.*[1] was used to predict the immunogenic potential of ADAMTS13 peptides at the population level. The 20 peptides identified elution studies (Table 3) (from this study and a previous study from our department (Sorvillo, *et al.*)[2]). These input sequences were parsed into overlapping 9-mer sequences (representing the actual length of the HLA DR binding groove) and each 9-mer subsequence was evaluated for potential binding with respect to a panel of nine common Class II HLA-DR alleles ("HLA allele families") using EpiMatrix. These nine "super-type" alleles represent over 95% of HLA-DR alleles of the human population.[3] The immunogenic potential of eluted peptides was assessed using JanusMatrix.[4] In brief, this tool searches HLA-binding peptides in the human proteome for matches to the TCR-facing residues of HLA-DR binding epitopes from an input sequence. Peptides that are cross-conserved (at the TCR face) are considered to be either tolerated or more likely to be recognized by regulatory T cells. Peptides that have less TCR-facing conservation are considered to be more likely recognized by effector T cells. Previous studies have shown that peptides that bind promiscuously to HLA-DR and that have extensive cross-conservation with highly prevalent peptides in the human proteome can be actively regulatory.

Supplementary results

Supplementary table 1. Representative list of 20 proteins with the highest score and number of unique peptides presented on HLA-DR (A) and HLA-DQ (B) of ADAMTS13 pulsed dendritic cells of donor 1. Most peptides are derived from endogenous proteins. The number of unique peptides observed for each protein is depicted in the right column. Three ADAMTS13-derived peptides were identified on HLA-DR of donor 1.

Supplementary figure 1. Number of unique peptides identified in the MS samples for HLA-DR and HLA-DQ. Panel A depicts the total number of unique peptides identified on HLA-DR and HLA-DQ of all studied donors. The total number of unique ADAMTS13-derived peptides is shown in panel B.

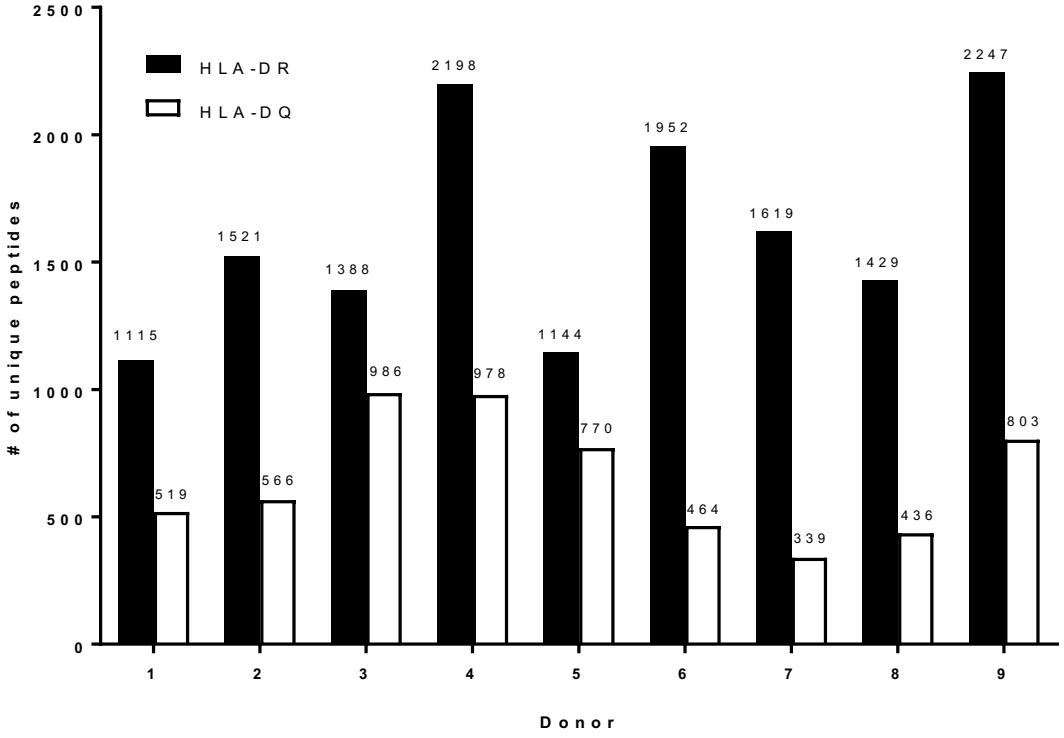
Supplementary table 1A

Protein	Score	# Unique Peptides
Integrin alpha-M OS=Homo sapiens GN=ITGAM PE=1 SV=2 - [ITAM_HUMAN]	163,03	39
Serotransferrin OS=Homo sapiens GN=TF PE=1 SV=3 - [TRFE_HUMAN]	158,10	27
Transforming growth factor-beta-induced protein ig-h3 OS=Homo sapiens GN=TGFBI PE=1 SV=1 - [BGH3_HUMAN]	110,10	26
HLA class I histocompatibility antigen, B-35 alpha chain OS=Homo sapiens GN=HLA-B PE=1 SV=1 - [1B35_HUMAN]	94,95	6
Lysosomal acid lipase/cholesteryl ester hydrolase OS=Homo sapiens GN=LIPA PE=1 SV=2 - [LICH_HUMAN]	90,27	10
Serum albumin OS=Homo sapiens GN=ALB PE=1 SV=2 - [ALBU_HUMAN]	86,10	24
Cathepsin B OS=Homo sapiens GN=CTSB PE=1 SV=3 - [CATB_HUMAN]	81,85	15
HLA class I histocompatibility antigen, B-59 alpha chain OS=Homo sapiens GN=HLA-B PE=2 SV=1 - [1B59_HUMAN]	72,16	0
Cathepsin Z OS=Homo sapiens GN=CTSZ PE=1 SV=1 - [CATZ_HUMAN]	67,05	13
Calreticulin OS=Homo sapiens GN=CALR PE=1 SV=1 - [CALR_HUMAN]	65,63	14
Transferrin receptor protein 1 OS=Homo sapiens GN=TFRC PE=1 SV=2 - [TFR1_HUMAN]	61,20	13
Cofilin-1 OS=Homo sapiens GN=CFL1 PE=1 SV=3 - [COF1_HUMAN]	60,08	11
60S ribosomal protein L22 OS=Homo sapiens GN=RPL22 PE=1 SV=2 - [RL22_HUMAN]	51,98	11
Peptidyl-prolyl cis-trans isomerase B OS=Homo sapiens GN=PPIB PE=1 SV=2 - [PPIB_HUMAN]	51,40	7
Macrophage mannose receptor 1 OS=Homo sapiens GN=MRC1 PE=1 SV=1 - [MRC1_HUMAN]	49,53	14
HLA class I histocompatibility antigen, B-8 alpha chain OS=Homo sapiens GN=HLA-B PE=1 SV=1 - [1B08_HUMAN]	49,48	2
Aminopeptidase N OS=Homo sapiens GN=ANPEP PE=1 SV=4 - [AMPN_HUMAN]	47,40	11
HLA class I histocompatibility antigen, B-44 alpha chain OS=Homo sapiens GN=HLA-B PE=1 SV=1 - [1B44_HUMAN]	35,33	1
Prolow-density lipoprotein receptor-related protein 1 OS=Homo sapiens GN=LRP1 PE=1 SV=2 - [LRP1_HUMAN]	35,12	11
Elongation factor 1-alpha 1 OS=Homo sapiens GN=EEF1A1 PE=1 SV=1 - [EF1A1_HUMAN]	34,89	10
A disintegrin and metalloproteinase with thrombospondin motifs 13 OS=Homo sapiens GN=ADAMTS13 PE=1 SV=1 - [ATS13_HUMAN]	5,65	3

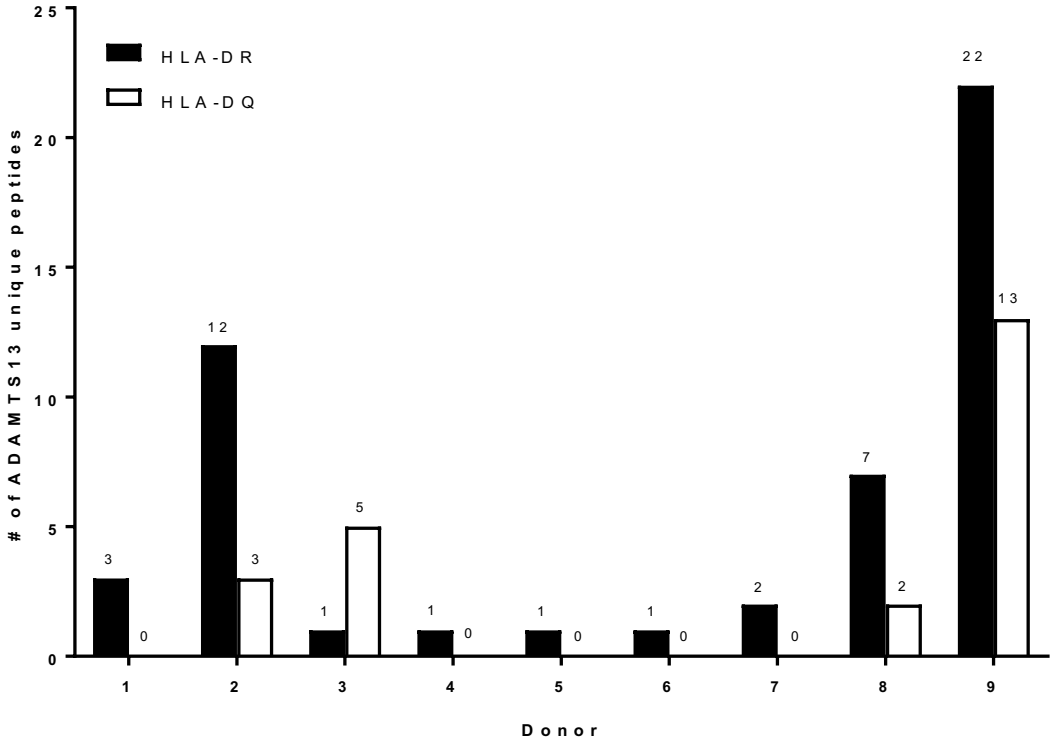
Supplementary table 1B

Protein	Score	# Unique Peptides
Heat shock protein HSP 90-beta OS=Homo sapiens GN=HSP90AB1 PE=1 SV=4 - [HS90B_HUMAN]	60,75	4
HLA class I histocompatibility antigen, B-35 alpha chain OS=Homo sapiens GN=HLA-B PE=1 SV=1 - [1B35_HUMAN]	55,27	7
Calreticulin OS=Homo sapiens GN=CALR PE=1 SV=1 - [CALR_HUMAN]	51,65	12
Lysosomal protective protein OS=Homo sapiens GN=CTSA PE=1 SV=2 - [PPGB_HUMAN]	51,44	8
Heat shock protein HSP 90-alpha OS=Homo sapiens GN=HSP90AA1 PE=1 SV=5 - [HS90A_HUMAN]	47,70	2
Peptidyl-prolyl cis-trans isomerase B OS=Homo sapiens GN=PPIB PE=1 SV=2 - [PPIB_HUMAN]	43,79	7
Serotransferrin OS=Homo sapiens GN=TF PE=1 SV=3 - [TRFE_HUMAN]	38,04	10
60S ribosomal protein L22 OS=Homo sapiens GN=RPL22 PE=1 SV=2 - [RL22_HUMAN]	36,60	5
HLA class II histocompatibility antigen, DR alpha chain OS=Homo sapiens GN=HLA-DRA PE=1 SV=1 - [DRA_HUMAN]	33,72	8
Phospholipase D3 OS=Homo sapiens GN=PLD3 PE=1 SV=1 - [PLD3_HUMAN]	31,72	7
HLA class I histocompatibility antigen, Cw-17 alpha chain OS=Homo sapiens GN=HLA-C PE=1 SV=1 - [1C17_HUMAN]	29,97	6
Actin, cytoplasmic 1 OS=Homo sapiens GN=ACTB PE=1 SV=1 - [ACTB_HUMAN]	27,58	10
40S ribosomal protein S17 OS=Homo sapiens GN=RPS17 PE=1 SV=2 - [RS17_HUMAN]	26,89	2
Serum albumin OS=Homo sapiens GN=ALB PE=1 SV=2 - [ALBU_HUMAN]	25,40	7
60S ribosomal protein L30 OS=Homo sapiens GN=RPL30 PE=1 SV=2 - [RL30_HUMAN]	23,85	4
Activated RNA polymerase II transcriptional coactivator p15 OS=Homo sapiens GN=SUB1 PE=1 SV=3 - [TCP4_HUMAN]	23,38	1
Vimentin OS=Homo sapiens GN=VIM PE=1 SV=4 - [VIME_HUMAN]	23,18	5
Histone H2B type 1-L OS=Homo sapiens GN=HIST1H2BL PE=1 SV=3 - [H2B1L_HUMAN]	21,87	8
HLA class II histocompatibility antigen, DRB1-15 beta chain OS=Homo sapiens GN=HLA-DRB1 PE=1 SV=2 - [2B1F_HUMAN]	21,57	6
60S ribosomal protein L38 OS=Homo sapiens GN=RPL38 PE=1 SV=2 - [RL38_HUMAN]	20,74	3

Supplementary figure 1A



Supplementary figure 1B



References

1. De Groot AS, Martin W. Reducing risk, improving outcomes: bioengineering less immunogenic protein therapeutics. Clin Immunol 2009;131(2):189–201.
2. Sorvillo N, van Haren SD, Kaijen PH, et al. Preferential HLA-DRB1*11-dependent presentation of CUB2-derived peptides by ADAMTS13-pulsed dendritic cells. Blood 2013;121(17):3502–3510.
3. Southwood S, Sidney J, Kondo A, et al. Several common HLA-DR types share largely overlapping peptide binding repertoires. J Immunol 1998;160(7):3363–3373.
4. Moise L, Gutierrez AH, Bailey-kellogg C, et al. The two-faced T cell epitope. 2013;(July):1–10.