Supplemental methods

Binding of peptides to HLA-DRB1* haplotypes

Cell-free REVEAL class II binding technology (ProImmune, www.proimmune.com).

This technology measures the ability of each peptide to stabilize the MHC-peptide complex. Detection is based on the presence or absence of the native conformation of the MHC-peptide complex, which is detected by a specific monoclonal antibody. Each peptide is given a score relative to a positive control peptide, which is a known T-cell epitope. The score is reported quantitatively as a percentage of the signal generated by the test peptide compared with the positive control peptide. The following positive control peptides were included: Influenza HA 306-318 (PKYVKQNTLKLAT) as the reference peptide for HLA-DRB1*0101, HLA-DRB1*0401, HLA-DRB1*0707 and HLA-DRB1*1101; sw Myoglobin 137-148 (LFRKDIAAKYKE) as the reference peptide for HLA-DRB1*0301; human MBP 84-102 (NPVVHFFKNIVTPRTPPPS) as the reference peptide for HLA-DRB1*1501

Cumulative Pan-Allele Proimmune REVEAL^{TM} binding score of peptide binding

Binding of dominant T-cell epitopes of FVIII, identified in E17 HLA-DRB1*1501 mice, to the 6 most common HLA-DRB1* haplotypes was analyzed using the REVEAL class II binding technology. The Cumulative Pan-Allele ProImmune REVEAL score

for each peptide was calculated by adding up the binding scores calculated for each of the 6 HLA-DRB1* haplotypes analyzed and dividing the sum by 6 (number of HLA-DRB1* haplotypes analyzed). Different colors corresponding to the contribution of each of the different HLA-DRB1* haplotypes tested were used for illustration (see Figure 6A).

The kinetics score for peptide binding

Off-rates and on-rates of peptide binding were analyzed for each peptide and calculated relative to the positive control peptides. The kinetics score for each peptide was calculated by dividing the relative off-rate by the relative on-rate for each HLA-DRB1* haplotype. The kinetics score is expressed in percent. The following positive control peptides were included: Influenza HA 306-318 (PKYVKQNTLKLAT) as the reference peptide for HLA-DRB1*0101, HLA-DRB1*0401, HLA-DRB1*0707 and HLA-DRB1*1101; sw Myoglobin 137-148 (LFRKDIAAKYKE) as the reference peptide for HLA-DRB1*0301; human MBP 84-102 (NPVVHFFKNIVTPRTPPPS) as the reference peptide for HLA-DRB1*1501.

Cumulative Pan-Allele kinetics score of peptide binding

The kinetics scores for peptide binding to the 6 most common HLA-DRB1* haplotypes were calculated as described above. The Cumulative Pan-Allele kinetics score for each peptide was calculated by adding up the kinetics scores calculated for each of the 6 HLA-DRB1* haplotypes analyzed and dividing the sum by 6 (number of HLA-DRB1* haplotypes analyzed). Different colors corresponding to the contribution

of each of the different HLA-DRB1* haplotypes tested were used for illustration, (see Figure 6B).

Analysis of the combinatorial diversity of the T-cell receptor β chain

The diversity of the T-cell receptor β chain was analyzed in $CD4^+$ T cells purified from spleen cells of naïve mice. Spleens were obtained from naïve humanized E17 HLA-DRB1*1501 mice and from naïve conventional E17 mice and spleen cells were prepared as described.[28] $CD4^+$ T cells were purified from spleen cells by magnetic cell sorting using mouse CD4 Dynabeads® (Invitrogen, Life Technologies). The repertoire of the T-cell receptor β chain was assessed by analyzing the combinational diversity of V-J gene rearrangements of the T-cell receptor β chain in genomic DNA. DNA was isolated from purified splenic $CD4^+$ T cells obtained from naïve mice and analyzed by ImmunID Technologies, Grenoble France, using an ImmunTraCkeR® test as described by Pascal et al.[32] The ImmunTraCkeR® test is based on a multiplex PCR that uses upstream specific primers for all functional members of a given TRBV gene family and downstream primers specific for TRBJ segments. The multiplex PCR allows the simultaneous detection and resolution of several TRB V–J rearrangements in the same PCR run. This technique has the capacity to detect about 100% of the potential 209 TRB VJ combinatorial rearrangements. The overall TRB VJ diversity of an mTRB repertoire of $CD4^+$ T cells is expressed as relative diversity compared with the theoretical maximum of 209 TRB VJ combinatorial rearrangements. The analyses were done using the Constel'ID® software from ImmunID Technologies.

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Table S1. FVIII peptide regions containing $CD4^+$ T cell epitopes identified in humanized E17 HLA-DRB1*1501 mice (human MHC-class II) and in conventional hemophilic E17 mice (murine MHC-class II).

human MHC-class II (HLA-DRB1*1501)		**murine MHC-class II**	
$FVIII^{83-103}$	TVVITLKNMASHPVSLHAVGV	$FVIII^{11-28}$	ELSWDYMQSDLGELPVDA
$FVIII^{227-247}$	AWPKMHTVNGYVNRSLPGLIG	$FVIII^{23-43}$	ELPVDARFPPRVPKSFPFNTS
$FVIII^{455-475}$	GEVGDTLLIIFKNQASRPYNI	$FVIII^{65-79}$	RPPWMGLLGPTIQAE
$FVIII^{521-541}$	PTKSDPRCLTRYYSSFVNMER	$FVIII^{101-118}$	VGVSYWKASEGAEYDDQT
$FVIII^{1382-1405}$	QANRSPLPIAKVSSFPSIRPIYLT	$FVIII^{524-544}$	SDPRCLTRYYSSFVNMERDLA
$FVIII^{1766-1786}$	EVEDNIMVTFRNQASRPYSFY	$FVIII^{602-622}$	QLEDPEFQASNIMHSINGYVF
$FVIII^{2006-2026}$	LHAGMSTLFLVYSNKCQTPLG	$FVIII^{770-787}$	DPWFAHRTPMPKIQNVSS
$FVIII^{2141-2161}$	NPPIIARYIRLHPTHYSIRST	$FVIII^{1016-1036}$	DGPSLLIENSPSVWQNILESD
		$FVIII^{1031-1051}$	NILESDTEFKKVTPLIHDRML
		$FVIII^{1202-1222}$	ETLIQENVVLPQIHTVTGTKN
		$FVIII^{1340-1360}$	WSKNMKHLTPSTLTQIDYNEK
		$FVIII^{1373-1393}$	CLTRSHSIPQANRSPLPIAKV
		$FVIII^{1595-1612}$	KDTILSLNACESNHAIAA
		$FVIII^{1706-1726}$	LWDYGMSSSPHVLRNRAQSGS
		$FVIII^{2069-2089}$	SWIKVDLLAPMIIHGIKTQGA
		$FVIII^{2135-2152}$	IKHNIFNPPIIARYIRLH
		$FVIII^{2195-2212}$	YFTNMFATWSPSKARLHL

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Table S2: On rates (t½ in hours) for binding of FVIII peptides to different human HLA-DRB1* haplotypes

Peptide	On rates (t1/2 in hours)					
	DRB1*0101	DRB1*1501	DRB1*0301	DRB1*0401	DRB1*1101	DRB1*0701
TVVITLKNMASHPVS	17,35	4,39	>250,00	15,32	30,49	8,05
ITLKNMASHPVSLHA	61,57	8,30	***	29,78	15,99	>120,00
KNMASHPVSLHAVGV	17,98	4,56	9,06	17,62	7,04	>120,00
AWPKMHTVNGYVNRS	0,43	72,00	***	2,34	28,96	27,22
KMHTVNGYVNRSLPG	2,95	28,00	***	4,44	10,66	3,72
TVNGYVNRSLPGLIG	5,02	18,00	***	6,67	59,97	43,59
GEVGDTLLIIFKNQA	>250,00	10,65	***	12,85	22,15	11,80
GDTLLIIFKNQASRP	7,79	5,41	9,18	9,45	15,85	7,54
LLIIFKNQASRPYNI	11,89	9,12	8,75	13,09	37,69	4,10
PTKSDPRCLTRYYSS	>250,00	2,19	>250,00	7,12	4,97	9,26
SDPRCLTRYYSSFVN	5,61	2,99	***	9,12	6,37	3,32
RCLTRYYSSFVNMER	2,67	15,94	***	19,64	5,52	11,10
QANRSPLPIAKVSSF	***	>120,00	***	0,87	10,40	>120,00
RSPLPIAKVSSFPSI	9,85	16,73	***	7,47	7,55	24,82
LPIAKVSSFPSIRPI	2,58	3,35	***	7,92	4,11	10,27
AKVSSFPSIRPIYLT	10,73	7,67	***	>120,00	10,44	5,90
EVEDNIMVTFRNQAS	5,48	21,09	***	8,58	15,08	3,94
DNIMVTFRNQASRPY	>250,00	9,19	>250,00	8,82	8,05	14,60
MVTFRNQASRPYSFY	17,60	18,01	***	19,00	19,97	>120,00
LHAGMSTLFLVYSNK	8,66	12,47	36,38	41,03	15,11	>120,00
GMSTLFLVYSNKCQT	19,63	6,56	3,62	15,93	10,48	59,47
TLFLVYSNKCQTPLG	65,86	24,90	>250,00	18,15	25,45	>120,00
NPPIIARYIRLHPTH	7,53	4,12	>250,00	14,48	5,34	8,85
IIARYIRLHPTHYSI	6,89	5,39	***	9,84	9,25	5,85
RYIRLHPTHYSIRST	7,13	3,29	***	9,11	5,86	8,65
Influenza HA 306-318	**5,77**	38,32	***	**5,87**	**10,75**	**6,03**
sw Myoglobin 137-148	17,78	***	**11,69**	7,50	***	5,41
human MBP 84-102	14,09	**4,40**	***	12,07	10,77	>120,00

On-rates of individual peptides were measured at 6 time points over a 48 hour period (HLA-DRB1*0101, HLA-DRB1*0301) or over a 24 hour period (HLA-DRB1*1501, HLA-DRB1*0401, HLA-DRB1*1101, HLA-DRB1*0701). The time period used was optimized for each haplotype. The maximum signal during assembly obtained for each individual peptide was set to 100%. The on - rates are expressed as t ½ (hours) values (time to half maximum value). Influenza HA306-318, sw Myoglobin 137-148 and human MBP were used as positive control peptides. Control peptide on-rates are indicated in bold for each HLA-DRB1* haplotype. Grey colored boxes indicate that the observation period was too short to reach an equilibrium binding for these specific peptides. An estimated value of 250h (for HLA-DRB1*0101, HLA-DRB1*0301) or 120h (for HLA-DRB1*1501, HLA-DRB1*0401, HLA-DRB1*1101, HLA-DRB1*0701) has been substituted for these peptides. *** no stable complex of HLA-DRB1* haplotype and peptide was formed.

Table S3. Off rates (t½ in hours) for binding of FVIII peptides to different human HLA-DRB1* haplotypes

Peptide	Off rates (t1/2 in hours)					
	DRB1*0101	DRB1*1501	DRB1*0301	DRB1*0401	DRB1*1101	DRB1*0701
TVVITLKNMASHPVS	17,59	>120,00	>120,00	>120,00	3,56	108,40
ITLKNMASHPVSLHA	16,58	>120,00	***	>120,00	>120,00	>120,00
KNMASHPVSLHAVGV	13,45	3,15	***	>120,00	>120,00	20,81
AWPKMHTVNGYVNRS	***	2,93	***	>120,00	1,68	>120,00
KMHTVNGYVNRSLPG	2,55	36,78	***	1,29	4,97	1,64
TVNGYVNRSLPGLIG	0,98	12,21	***	90,52	>120,00	5,25
GEVGDTLLIIFKNQA	40,47	3,66	***	16,97	>120,00	2,26
GDTLLIIFKNQASRP	54,98	27,59	0,42	>120,00	7,90	2,12
LLIIFKNQASRPYNI	51,60	15,33	0,66	>120,00	>120,00	2,34
PTKSDPRCLTRYYSS	23,26	2,28	5,88	0,45	92,90	1,69
SDPRCLTRYYSSFVN	***	22,60	***	***	2,87	1,07
RCLTRYYSSFVNMER	2,52	30,34	***	>120,00	>120,00	1,22
QANRSPLPIAKVSSF	***	2,31	***	***	***	1,29
RSPLPIAKVSSFPSI	20,72	21,54	***	112,60	4,36	28,70
LPIAKVSSFPSIRPI	2,14	28,48	***	1,20	>120,00	9,24
AKVSSFPSIRPIYLT	***	36,23	***	***	44,75	2,06
EVEDNIMVTFRNQAS	2,94	1,58	***	6,79	41,24	2,03
DNIMVTFRNQASRPY	36,17	40,01	18,21	>120,00	2,55	2,72
MVTFRNQASRPYSFY	9,80	14,32	***	>120,00	74,37	3,99
LHAGMSTLFLVYSNK	***	3,03	>120,00	***	19,77	8,29
GMSTLFLVYSNKCQT	24,23	20,67	9,15	>120,00	0,92	>120,00
TLFLVYSNKCQTPLG	8,18	34,67	>120,00	>120,00	43,92	>120,00
NPPIIARYIRLHPTH	9,63	38,56	87,15	>120,00	43,29	3,08
IIARYIRLHPTHYSI	29,45	>120,00	***	>120,00	3,29	>120,00
RYIRLHPTHYSIRST	25,68	65,03	***	>120,00	92,54	>120,00
Influenza HA 306-318	**40,70**	11,91	***	**>120,00**	**>120,00**	**>120,00**
sw Myoglobin 137-148	13,35	***	**>120,00**	>120,00	***	2,23
human MBP 84-102	34,02	**96,93**	***	>120,00	54,37	>120,00

Off rates of individual peptides were measured at 6 time points over a 24h period at 37°C. The off-rates are calculated based on the maximal signal obtained for each individual peptide (t=0h). Influenza HA306-318, sw Myoglobin 137-148 and human MBP were used as positive control peptides. Control peptide off-rates are indicated in bold for each each HLA-DRB1* haplotype. Grey colored boxes indicate that the observation period was too short to calculate these values accurately. An estimated value of 120h has been substituted for these peptides.

*** no stable complex of HLA-DRB1* haplotype and peptides was present at the start of analysis.