Supporting Information

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SI Methods

Protein Crystallization. All crystals for data collection were produced by the hanging-drop vapor-diffusion method. The three IA^{g7}–insulin peptide complexes and DQ8-8E9E11ss were crystallized at room temperature at a concentration of 7 mg/mL. The IA^{g7}–8E9E crystals were grown from 20% PEG3350, 100 mM sodium citrate at pH 5.0, and 5% isopropanol. IA^{g7}–8E9E6ss crystals were grown from 15% PEG4000, 10 mM sodium citrate at pH 5.0, and 100 mM $MgCl_2$. IA^{g7}–8G9E crystals were grown from 18% PEG20000 and 100 mM sodium citrate at pH 5.0. DQ8-8E9E11ss crystals were grown from 15% PEG3350, Tacimate pH 4.5. All of the crystals of the MHC II–insulin peptide complexes were cryoprotected by well solution plus 25% glycerol.

Data Collection, Data Processing, and Structural Analysis. All diffraction datasets were collected at synchrotron beamline ID-24C at the Advanced Photon Source, Argonne National Laboratory using the Pilatus detector. The collected data were processed with HKL2000 package (1), the structures were solved by molecular replacement method using Phaser (2) software and further refined by refmac5 (3), and rebuilding of the structure was performed by Coot (4). Data collection and refinement statistics are shown in Table S2. Molecular superimpositions were performed with Swiss PDBViewer (5). Graphical representations of structures were constructed with PyMol (Schrodinger) and Discovery Studio 3 (Accelrys).

The atomic coordinates and structure factors have been deposited in the Research Collaboratory for Structural Bioinformatics Protein Data Bank, https://www.rcsb.org (PDB ID codes are shown in Table S2).

1. Otwinowski Z, Minor W (1997) Processing of X-ray diffraction data collected in oscillation mode. *Methods Enzymol* 276:307–326.
2. McCoy AJ, et al. (2007) Phaser crystallographic software. *J Appl Crystallogr* 40:658–674.
3. Murshudov GN, Vagin AA, Dodson EJ (1997) Refinement of macromolecular structures by the maximum-likelihood method. *Acta Crystallogr D Biol Crystallogr* 53:240–255.
4. Emsley P, Lohkamp B, Scott WG, Cowtan K (2010) Features and development of Coot. *Acta Crystallogr D Biol Crystallogr* 66:486–501.
5. Guex N, Peitsch MC (1997) SWISS-MODEL and the Swiss-PdbViewer: An environment for comparative protein modeling. *Electrophoresis* 18:2714–2723.

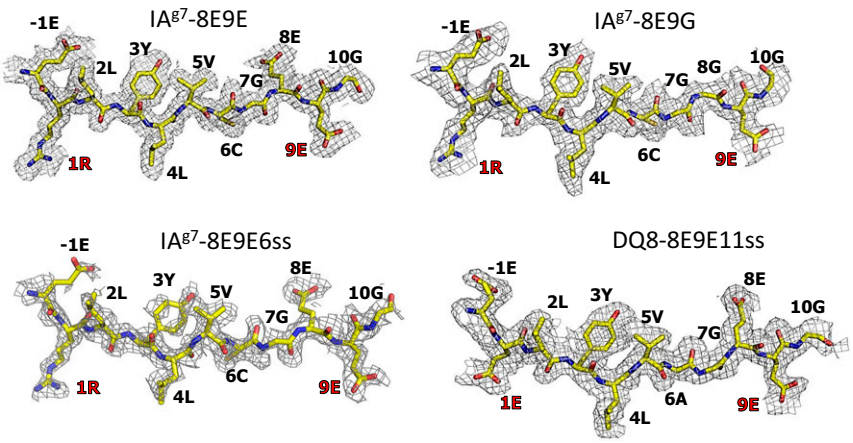

Fig. S1. Peptide omit electron density maps for the IA^{g7} and DQ8 structures. Shown are simulated annealing composite peptide omit 2Fo−Fc electron density maps (gray mesh) contoured at 1 σ within a 2 Å radius of the mutated insulin peptide in the four structures reported here.

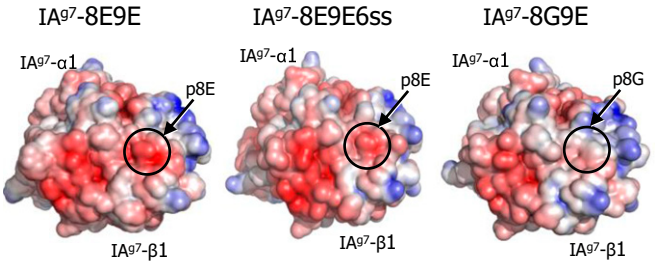

Fig. S2. The solvent-accessible surface electrostatic potential of the 8E9E, 8E9E6ss, and 8G9E mutant peptides bound to IA^{g7}. The IA^{g7}–peptide surface electrostatic potential is shown on its TCR-accessible interface (blue, positive; red, negative). Orientations are the same as in Figs. 4*C* and 5*E*. The contribution from the p8 amino acid is circled and labeled.

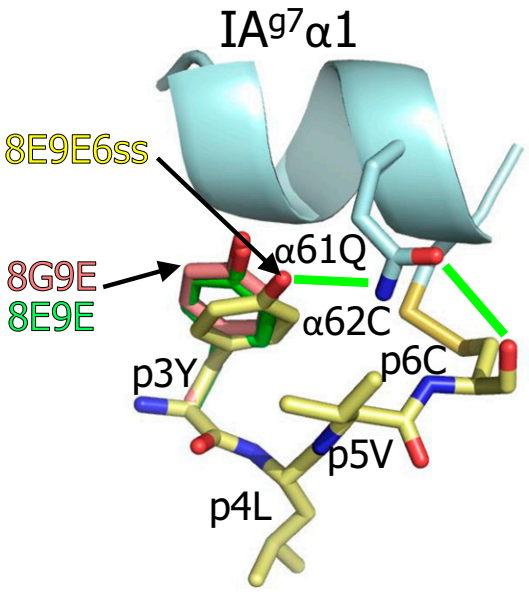

Fig. S3. Structural differences between the IA^{g7}–8E9E and IA^{g7}–8E9E6ss complexes may explain their different potencies for the I.29 T cell. A top view is shown of a portion of the IA^{g7} α1 helix from the IA^{g7}–8E9E6ss with side chains of 61Q and 62C (carbons, cyan) along with a wireframe representation of the 8E9E6ss peptide (carbons, yellow) from p3Y to p6C in the same structure. H bonds are shown in green. Also shown superimposed are the side chains of p3Y from IA^{g7}–8E9E (carbon, green) and IA^{g7}–8G9E (carbon, light red).

Table S1. Properties of the TCRs of the mouse and human B:9–23-specific CD4 T cells used in these studies

	V alpha domain*					V beta domain*				
T cell	Vα (TRAV)	CDR1†	CDR2†	CDR3‡	Jα	Vβ (TRBV)	CDR1†	CDR2†	CDR3‡	Jβ
Mouse Type A										
I.29	15 (10)	DTASSY	DIRSNV	AASPSNSGGSNYKLT	53	2 (1)	NSQYPW	LRSPGD	TCSAGLGYEQY	2–7
PCR1–10	13.1 (5D-4)	DSASNY	DIRSNM	AASKTGGNNKLT	56	11 (16)	ISGHSA	FRNQAP	ASSLDGGQGLEQY	2–7
12–4.1	13.1 (5D-4)	DSASNY	DIRSNM	AASGANSGGSNYKLT	53	2 (1)	NSQYPW	LRSPGD	TCSPGLGNEQY	2–7
AS150	10.8 (13-1)	STTLNS	RLFYNP	AISSGSWQLI	22	2 (1)	NSQYPW	LRSPGD	TCSADQNSYNSPLY	1–6
Mouse Type B										
8F10	13.1 (5D-4)	DSASNY	DIRSNM	AASRRGSGGSNYKLT	53	8.2 (13-2)	TNNHNN	SYGAGS	ASGGLGGDEQY	2–7
8–1.1	13.1 (5D-4)	DSASNY	DIRSNM	AASKTGGNNKLT	56	12 (15)	VSGHND	FRSKSL	ASSLGWGDEQY	2–7
12–4.4	13.1 (5D-4)	DSASVY	DIRSNM	AASASGGSNTKLT	53	12 (15)	VSGHND	FRSKSL	ASSPGQGTTLY	1–3
AS91	13.2 (5D)	DSASVY	DIRSNM	SRGNNNRIF	31	1 (5)	HLGHNA	YNLKQL	ASSQLGGLDTQY	2–5
Human Type A										
T1D-3	3 (17)	TSINNL	LIRSNE	ATDAGYNQGGKLI	23	5.1 (5-1)	ISGHRS	YFSETQ	ASSAGNTIY	1–3
T1D-4	8 (13.1)	DSASNY	DIRSNV	AASKASNTGKLI	4	8.3 (12-5)	ILGHNT	FRNRAP	ASLKATDTQY	2–3
T1D-10	2.2 (12-3)	NSAFQY	YSSGNK	ATAYGQNFV	26	7.1 (4-1)	HMGHRA	SYEKLS	ASSRGGGNTGELF	2–2

*The Vα, Jα, Vβ, and Jβ elements as well as the CDR loop sequences are shown.
†CDR1 and CDR2 sequences are the six amino acids at the tips of these loops.
‡CDR3 sequences are amino acids between the conserved Cys in the V element and the conserved Phe in the FGXG motif of the J element.

Table S2. Crystallographic data and refinement statistics

	IA^{g7}	IA^{g7}	IA^{g7}	HLA-DQ8
	8E9E	8E9E6ss	8G9E	8E9E11ss
Data collection and refinement	PDB ID code 6BLQ	PDB ID code 6BLR	PDB ID code 6BLX	PDB ID code 5UJT
Data collection				
Space group	$P2_1$	$C222_1$	$P2_1$	$P2_12_12_1$
Cell parameters				
a	39.27	89.74	39.35	72.038
Dimentions, Å b	111.73	111.38	112.63	138.767
c	62.17	95.28	62.18	159.735
α	90.00	90.00	90.00	90.00
Angles, ° β	107.85	90.00	107.36	90.00
γ	90.00	90.00	90.00	90.00
Resolution range, Å	50–1.80	50–1.96	50–2.32	100–1.94
R_{sym} or R_{merge}*,†	0.09 (0.53)	0.07 (0.48)	0.08 (0.46)	0.10 (0.74)
$I/\sigma I$*	12.5 (1.77)	11.7 (1.9)	8.7 (1.45)	6.4 (1.2)
Completeness,* %	99.8 (93.4)	74.6 (72.1)	99.7 (96.2)	99.6 (98.6)
Redundancy*	5.8 (4.4)	4.5 (3.8)	3.2 (2.5)	2.0 (1.99)
Refinement				
Resolution range, Å	50–1.80	47.6–1.96	40.8–2.32	79.86–1.94
No. reflections	42,563	24,965	20,374	118,542
Rwork‡	15.97	16.26	17.18	20.90
Rfree‡	19.72	24.41	21.85	23.75
No. atoms	3,485	3,451	3,398	10,092
Protein	3,073	3,067	3,085	9,285
Ligand/ion	42	N/A	42	162
Water	370	384	271	645
B factors				
Protein	24.80	28.11	25.81	30.47
Ligand/ion	53.97	N/A	52.16	50.10
Water	35.33	35.20	28.72	34.79
rmsd				
Bond lengths, Å	0.019	0.007	0.019	0.008
Bond angles, °	1.950	0.900	1.948	1.098

*All data (outer shell).
†Rmerge = $\Sigma(|I - \langle I \rangle|)/\Sigma(I)$.
‡Rwork/Rfree = $\Sigma||F_o| - |F_c||/\Sigma|F_o|$. Rfree was calculated from a set of ~5% of the total reflections randomly chosen and set aside.