Supporting Information

Stadinski et al. 10.1073/pnas.1006545107

IA^{g7} Insulin B:11–20(19A) Register #1

Leader	Peptide	Linker	IA^{g7} β-chain
PGTEGQ	**LREALYLVAE**	QAGGGSLVPRGSGGGGS	ERHFVHQFK

IA^{g7} Insulin B:12–21(19A) Register #2

Leader	Peptide	Linker	IA^{g7} β-chain
PGTEGQ	**VRALYLVAGE**	QAGGGSLVPRGSGGGGS	ERHFVHQFK

IA^{g7} Insulin B:13–22(19A) Register #3

Leader	Peptide	Linker	IA^{g7} β-chain
PGTEGQ	**ERLYLVAGEE**	QAGGGSLVPRGSGGGGS	ERHFVHQFK

IA^{g7} Insulin B:14–23(19A) Register #4

Leader	Peptide	Linker	IA^{g7} β-chain
PGTEGQ	**ARYLVAGERE**	QAGGGSLVPRGSGGGGS	ERHFVHQFK

Fig. S1. Baculovirus constructions for optimizing registers 1–4. Amino acid sequences (bold letters) of insulin B-chain analog minimal peptides are shown in the context of the baculovirus constructions. The sequence (nonbolded letters) of the linker attaching the peptide to the N-terminal end the IA^{g7} β-chain is also shown as well as the C-terminal end of the leader peptide. The substitutions of p1R/p9E are shown in red. The change of B19Cys to Ala is underlined. In all four constructs, a Gln was added to the end of the leader peptide to ensure proper signal peptidase cleavage at the preceding Gly.

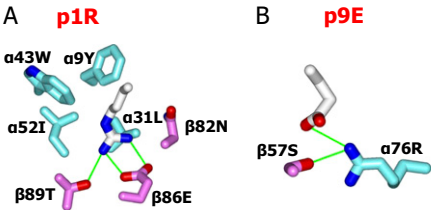

Fig. S2. Optimal amino acids for the p1 and p9 pockets of IA^{g7}. (*A*) Details of the p1 pocket of IA^{g7} with a bound HEL11-25 peptide (PDB ID code 1FJ3). The side chain of an Arg at p1 of the HEL peptide is shown filling the pocket with its guanidinium group forming a slat bridge to β86Glu of IA^{g7}. (*B*) Details of the p9 pocket of IA^{g7} with a bound GAD207-220 peptide (PDB ID code 1ES0). The side chain of glutamic acid at p9 of the GAD peptide is shown filing the pocket making a salt bridge α76Arg, which is unique to IA^{g7}.

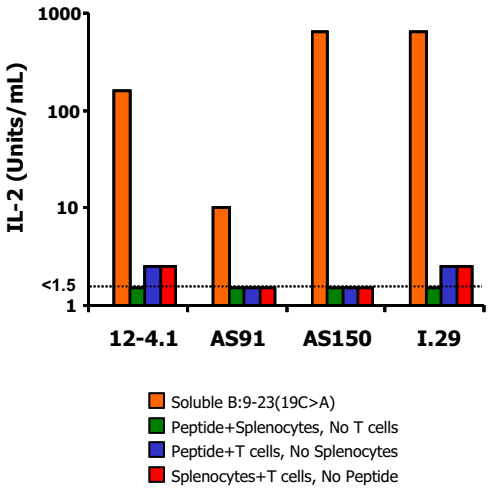

Fig. S3. Four B:9–23 reactive T cells tolerate a Cys-to-Ala substitution at insulin B19. Four anti-insulin (B:9–23) reactive T-cell hybridomas respond to soluble insulin B:9–23(C19A). The BDC 12-4.1, AS91, AS150, and I.29 T-cell hybridomas were stimulated with the insulin B:9–23 or the insulin B:9–23(C19A) peptide (50 μg/mL) along with NOD spleen cells (1 × 106/well). Stimulation was assessed by the production of IL-2 determined by an HT-2 bioassay. Results are the average from duplicate wells from one experiment.

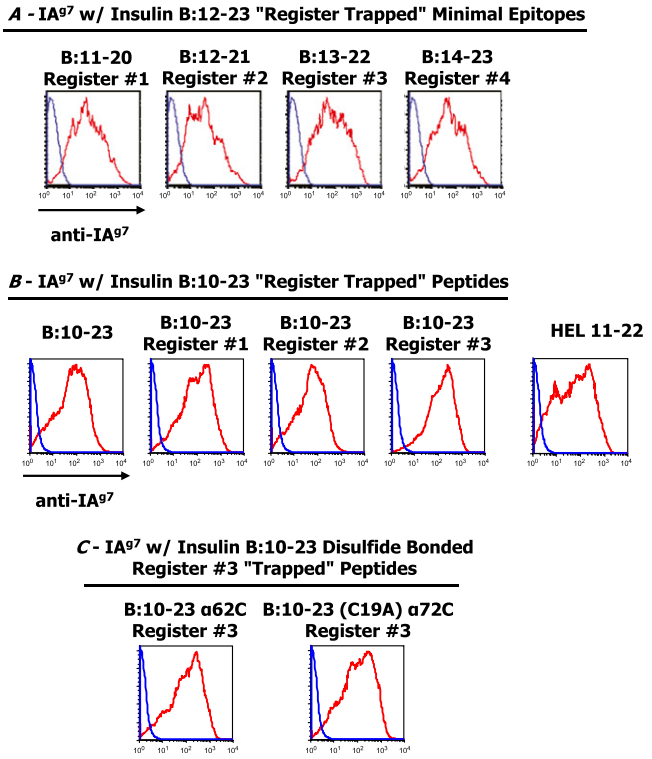

Fig. S4. All of the IA^{97} complexes containing various versions of insulin peptides express well on the surface of infected insect cells. Sf9 insect cells expressing the various IA^{97}-linked peptide complexes were stained with anti-IA^{97} Ab (OX6-PE). Surface expression was evaluated by flow cytometry comparing infected insect cells (red) to uninfected insect cells (blue). (*A*) Minimum (B:12–23) epitopes for the 12-4.1 T cell. (*B*) Extended (B:10–23) epitopes. (*C*) Extended epitopes with engineered disulfides.

T cell	TCR Chain	TCR V Region		TCR J Region	CDR3		
		Arden	IMGT	IMGT	V	Junction	J
BDC 12-4.1	α	Vα13	AV13S1	TRAV5D-4	TRAJ53*01	CAAS SGA	NSGGSNYKLTFG
	β	Vβ2	BV2S1	TRBV1	TRBJ2-7*01	CTCS PGLGK	EQYFG
AS91	α	Vα13	-	TRAV5-1	TRAJ31*01	CS RGN	NNRIFFG
	β	Vβ1	BV6S1A1	TRBV5	TRBJ2-5*01	CASS QLGGL	DTQYFG
AS150	α	Vα10	AV10S8	TRAV13-1	TRAJ22*01	CAI	SSGSWQLIFG
	β	Vβ2	BV2S1	TRBV1	TRBJ1-6*01	CTCS ADQ	NSYNSPLYFA
I.29	α	Vα15	AV15S1	TRAV10	TRAJ53*01	CAAS PS	NSGGSNYKLTFG
	β	Vβ2	BV2S1	TRBV1	TRBJ2-7*01	CTCS AGLG	YEQYFG

Fig. S5. The TCRs expressed by four independent anti-insulin B:9–23-specific T cells. cDNA was prepared from four independent insulin B-chain-responsive T-cell hybridomas and used as template in a PCR with Vα- and Vβ-specific 5′ primers and either a Cα or Cβ 3′ primer. The PCR fragment was sequenced to determine the Vα/Jα or Vβ/Jβ gene segments used as well as the sequence of the Vα and Vβ CDR3 loops. Shown are the common Vα and Vβ names as well as the genes used as defined by both the Arden et al. (1) and ImMunoGeneTics (IMGT) (2) nomenclatures. The TCR J region segments are shown using the IMGT nomenclature.

1. Arden B, Clark SP, Kabelitz D, Mak TW (1995) Mouse T-cell receptor variable gene segment families. *Immunogenetics* 42:501–530.
2. Giudicelli V, et al. (2006) IMGT/LIGM-DB, the IMGT comprehensive database of immunoglobulin and T cell receptor nucleotide sequences. *Nucleic Acids Res* 34(Database issue): D781–D784.

IA^{g7} HEL 11–22

Leader	Peptide	Linker	IA^{g7} β-chain
PGTEG	**MKRHGLDNYRGY**	GGGGSLVPRGSGGGGS	ERHFVHQFK

IA^{g7} Insulin B:10-23

Leader	Peptide	Linker	IA^{g7} β-chain
PGTEG	**HLVEALYLVCGERG**	AGGGGSLVPRGSGGGGS	ERHFVHQFK

IA^{g7} Insulin B:10–23 Register #1

Leader	Peptide	Linker	IA^{g7} β-chain
PGTEG	**HLREALYLVCEERG**	AGGGGSLVPRGSGGGGS	ERHFVHQFK

IA^{g7} Insulin B:10–23 Register #2

Leader	Peptide	Linker	IA^{g7} β-chain
PGTEG	**HLVRALYLVCGERG**	AGGGGSLVPRGSGGGGS	ERHFVHQFK

IA^{g7} Insulin B:10–23 Register #3

Leader	Peptide	Linker	IA^{g7} β-chain
PGTEG	**HLVERLYLVCGEEG**	AGGGGSLVPRGSGGGGS	ERHFVHQFK

IA^{g7} Insulin B:10–23 19A Register #3 P11 Cys

Leader	Peptide	Linker	IA^{g7} β-chain
PGTEG	**HLVERLYLVAGEEG**	CGGGGSLVPRGSGGGGS	ERHFVHQFK

Fig. S6. Amino acid sequences (bold letters) of the hen egg lysozyme (HEL) 11–22 peptide and N-terminally extended insulin B-chain peptides are shown in the context of the baculovirus constructions. The sequences of the C-terminal end of the signal peptide and the linker attaching the peptide to the N-terminal end the IA^{g7} β-chain are also shown. The substitutions to create p1R/p9E are shown in red. In the last construct a change of B19Cys (position p6) to Ala is underlined, and the Cys replacing the Ala at the first position of the linker (equivalent to peptide p11, orange).