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Title	Molecular Basis for Herpesvirus Entry Mediator Recognition by the Human Immune Inhibitory Receptor CD160 and Its Relationship to the Cosignaling Molecules BTLA and LIGHT
Author(s)	Kojima, Rieko; Kajikawa, Mizuho; Shiroishi, Mitsunori et al.
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Supplemental Table 1. Kinetics parameters for the interactions between CD160 or BTLA and HVEM compared with those of other protein-protein interactions.

Analyte	Immobilized	k_{on} $\times 10^5 (M^{-1}s^{-1})$	k_{off} (s^{-1})	$K_{d,kin}$ (μM)	$K_{d,eq}$ (μM)
CD160 (I27-S159)	HVEM	3.4	0.044	0.13	0.34 ± 0.01
		*0.72	*0.012	*0.17	*ND
BTLA (S33-D135)	HVEM	6.2	0.074	0.12	0.29 ± 0.01
		*ND	*ND	*ND	*0.25
Other protein-protein interactions		k_{on} $\times 10^5 (M^{-1}s^{-1})$	k_{off} (s^{-1})	K_d (μM)	References ^a
E-selectin	ESL	0.48	2.7	56	1
L-selectin	GlyCAM-1	>1	>10	108	2
P-selectin	PSGL-1	44	1.4	0.32	3
LILRB1D1D2	UL18	1.4	0.0028	0.0021	4
LILRB1D1D2	HLA-B35	5.0	3.7	7.4	5
LILRB1D1D2	HLA-Cw4	6.3	5.0	8.1	5
KIR2DL3	HLA-Cw7/DS11	2.1	1.1	5.2	6
CD8 $\alpha\alpha$	MHC class I	≥ 1.0	≥ 18	~ 200	7
CD22	CD45	≥ 1.5	≥ 18	117	8
CD80	CTLA-4	9.4	0.43	0.46	9
CD80	CD28	6.6	1.6	2.4	9
IgE-Fc	sFcϵRIα	2.5	6.5	0.0054	10
Fc γ RIIa,IIb,III	hFc1	3.8–4.4	0.31–0.69	0.72–1.9	11
TCR	Peptide-MHC	0.009–0.2	0.01–0.1	1–90	12, 13

The $K_{d,eq}$ values were obtained from the equilibrium analysis.

The $K_{d,kin}$ values were calculated from the simple 1:1 Langmuir binding model.

* Values were reported previously¹⁴ and are shown here for comparison. ND, not determined.

^aReferences

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mediator to its ligands through downregulation and direct competition. *J Virol* **84**, 11646-60.

Supplemental Figure 1. The MALDI-TOF mass spectrometric analysis of the extracellular Ig-V set domain of CD160h.

(A) MALDI-TOF mass spectrometric analysis of the purified CD160h Ig-V set domain expressed in HEK293 GNTI⁻ cells (Figure 1A, mixture of arrowheads 3-6) shows a molecular mass consistent with a monomer. The molecular mass of 18145.7 was detected. This molecular mass indicates the monomer Ig-V set domain of CD160h (estimated molecular weight; 15904.8) with two sugar modifications (Man5GlcNAc2) derived from HEK293S GNTI⁻ cells.

(B) The extracellular Ig-V set domain of CD160 expressed in *E. coli* and refolded *in vitro* (Supplemental Figure 2A, arrowhead) also showed the monomer molecular mass. A molecular mass of 14819.7, similar to the estimated molecular weight 14795.7, was detected.

Supplemental Figure 2. The extracellular Ig-V set domain of CD160, expressed in *E. coli* and refolded *in vitro*, is also monomeric.

(A) The inclusion bodies of the extracellular Ig-V set domain of CD160 expressed in BL21(DE3)plysS cells was refolded and purified by gel filtration chromatography (Superdex 75 26/60). The CD160 Ig-V set domain eluted in the 15 kDa range (Arrowhead).

(B) The CD160 Ig-V set domain purified in (A) was denatured with SDS sample buffer, with or without DTT, and resolved in a 15% SDS-PAGE gel followed by Coomassie Brilliant Blue (CBB) staining.

Supplemental Figure 3. DNTB assay of the refolded CD160.

DNTB assay of the extracellular Ig-V set domain of CD160, expressed in *E. coli* and refolded *in vitro* (Supplemental Figure 2A, arrowhead). A standard SH (reduced glutathione) calibration

curve with error bars is shown. Several concentrations of reduced glutathione were mixed with DNTB, and the OD_{412} values were measured. The curve-fitting equation is $Y = -5.2 \times 10^{-3} + 0.4 \times 10^{-3} X$; the correlation coefficient obtained is $R^2 = 0.998$. The OD_{412} values for 150 μ M and 200 μ M CD160 were measured three times at each concentration, and the estimated free SH concentrations are indicated. From these data, we conclude that refolded CD160 contains one non-paired thiol group.

Supplemental Figure 4. Binding responses of CD160 and BTLA to HVEM.

The purified, biotinylated form of either HVEM or BSA (negative control) was immobilized on a streptavidin-coupled CM5 sensor chip, and different concentrations of purified (A) CD160 and (B) BTLA were injected onto the chip. Analyte concentrations were the same in (A) and (B) (2-fold serial dilutions, 3 μ M highest concentration). The solid lines indicate HVEM responses, and the dotted lines indicate BSA responses.

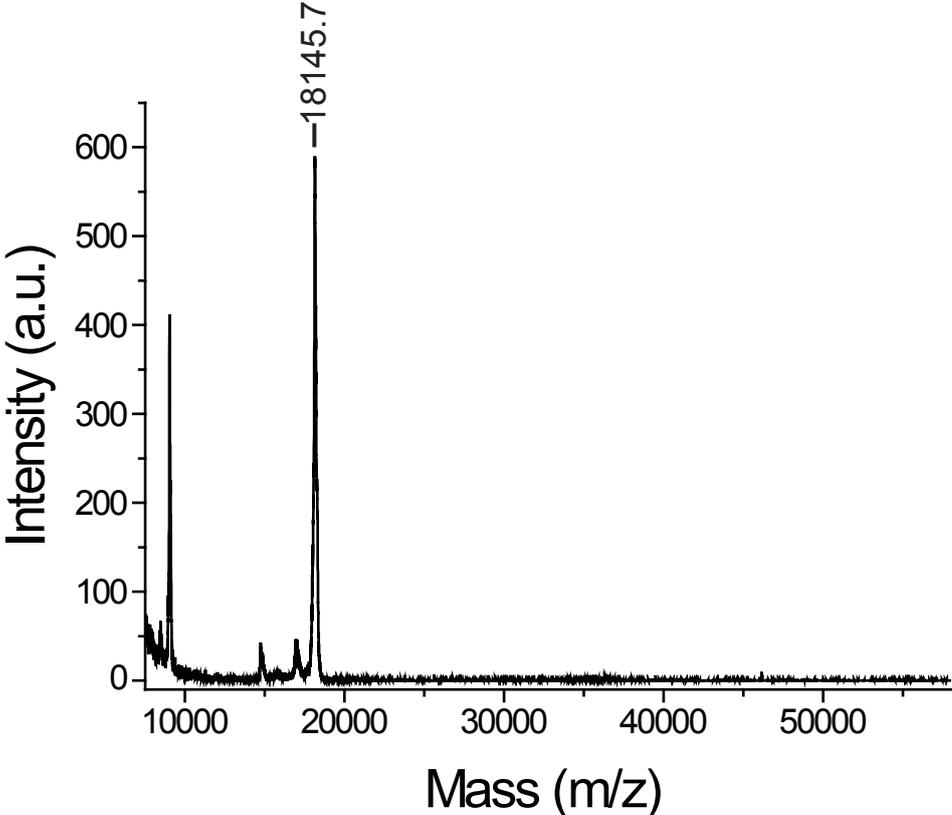
Supplemental Figure 5. Purification of BTLA Ig-V set domain.

(A) The inclusion bodies containing the BTLA Ig-V set domain (S33-D135) expressed in BL21(DE3)pLysS cells were refolded and purified by gel filtration chromatography (Superdex 75 26/60). The eluted BTLA (Arrowheads 1-6) was concentrated and used for SPR analysis. Arrowheads 1-6 indicate the fraction numbers.

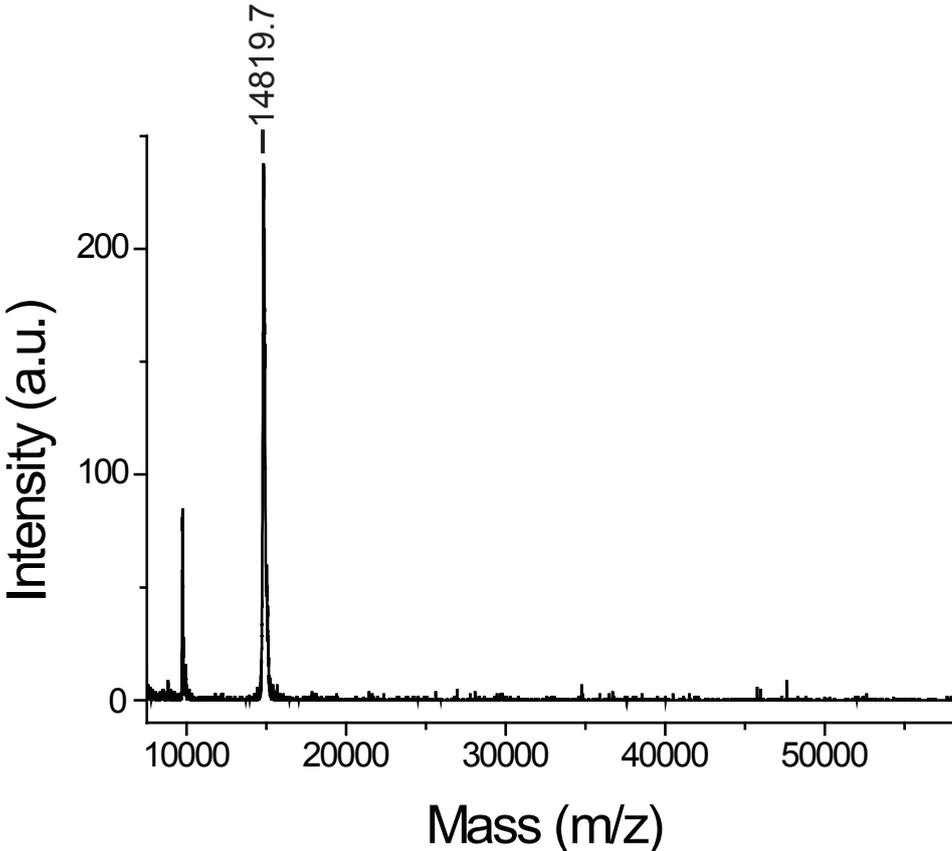
(B) BTLA purified in (A) was denatured with SDS sample buffer, mixed with DTT, and resolved on a 15% SDS-PAGE gel followed by CBB staining. The numbers 1-6 on the top of the membrane are the fractions 1-6 shown in (A). M indicates the protein size marker.

Supplemental Figure 1

A

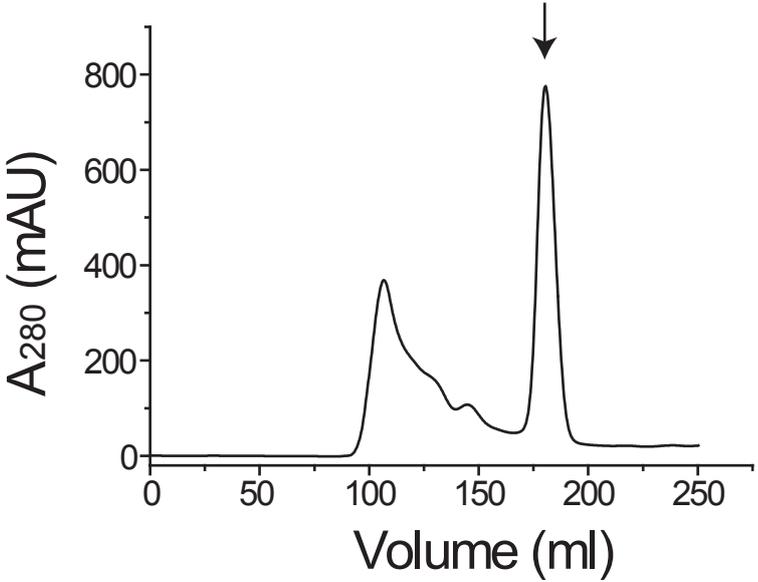


B

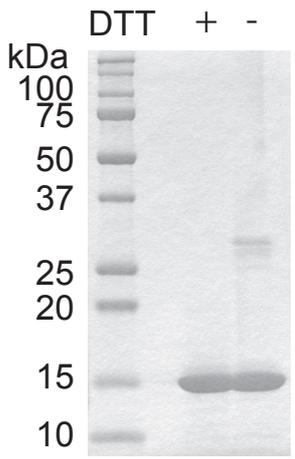


Supplemental Figure 2

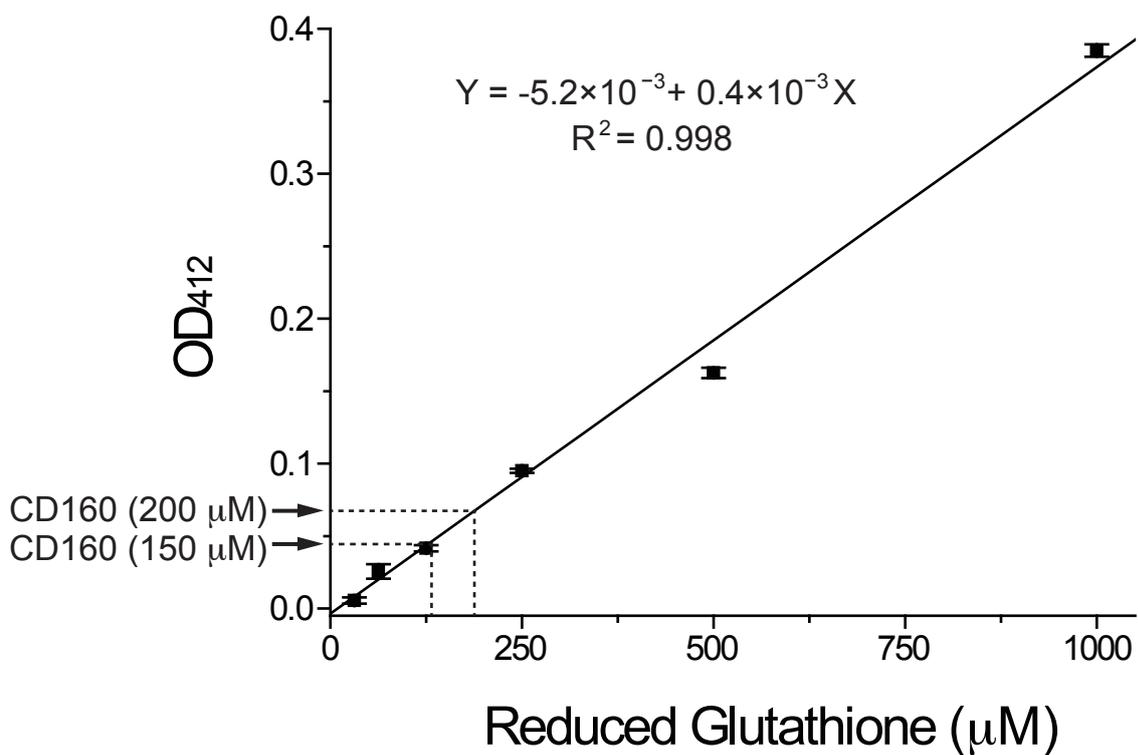
A



B



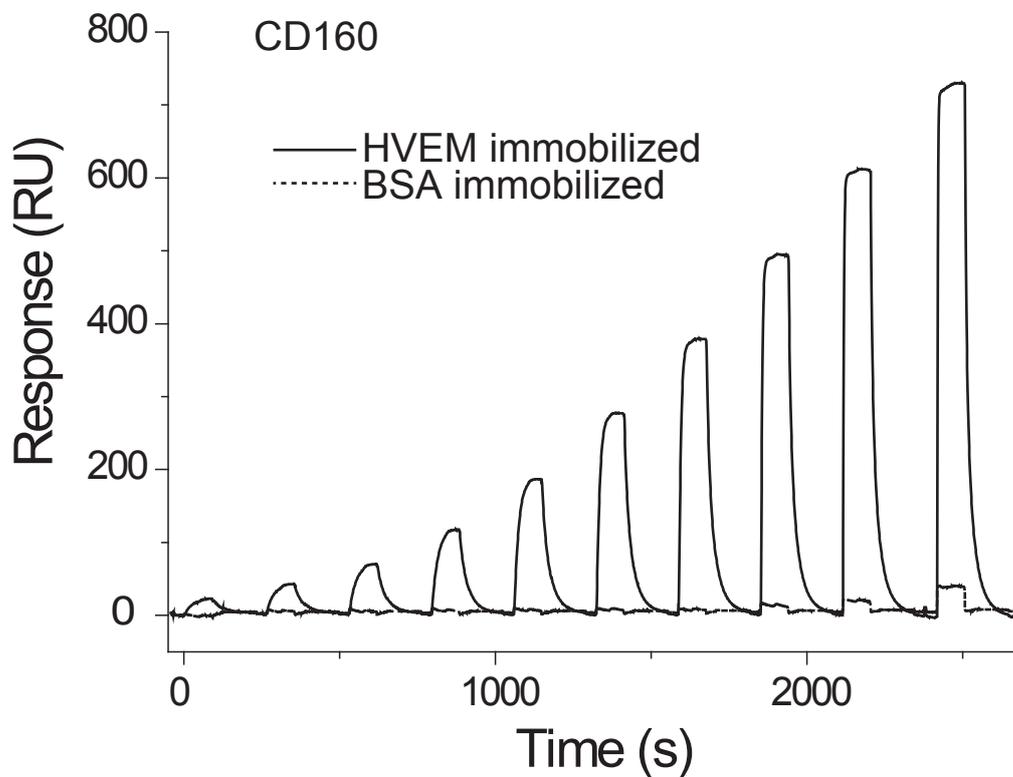
Supplemental Figure 3



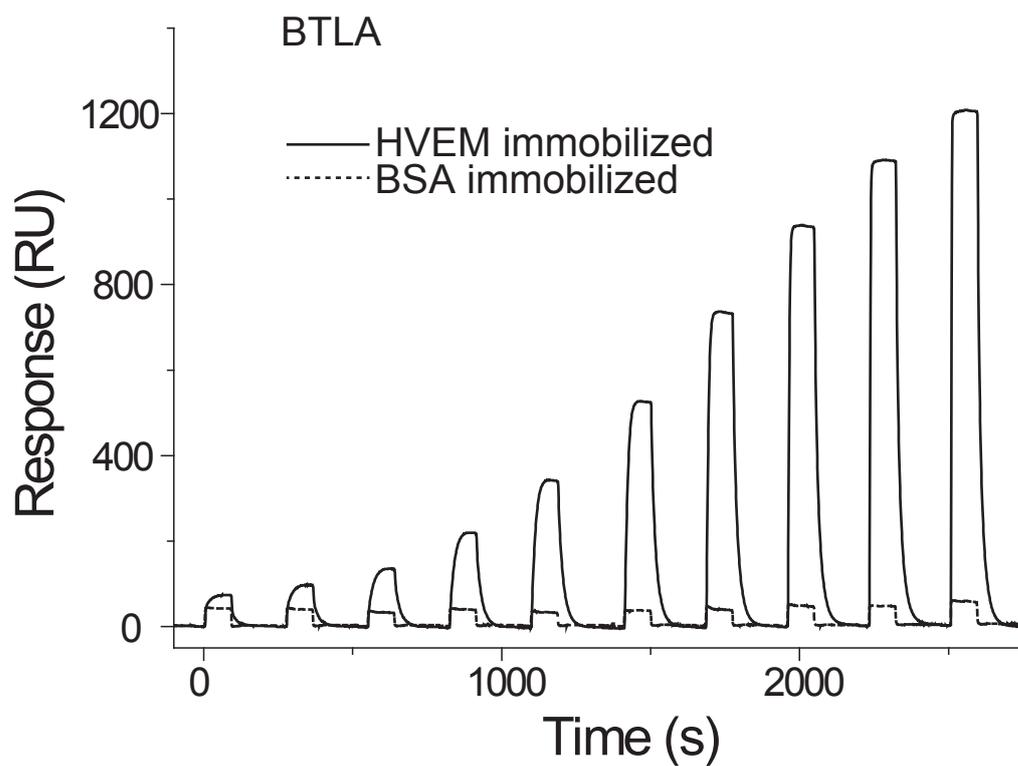
CD160 conc. (μM)	OD ₄₁₂	estimated free SH conc. (μM)
150	0.050 / 0.048 / 0.045	132 ± 6.3
200	0.067 / 0.073 / 0.069	187 ± 7.6

Supplemental Figure 4

A

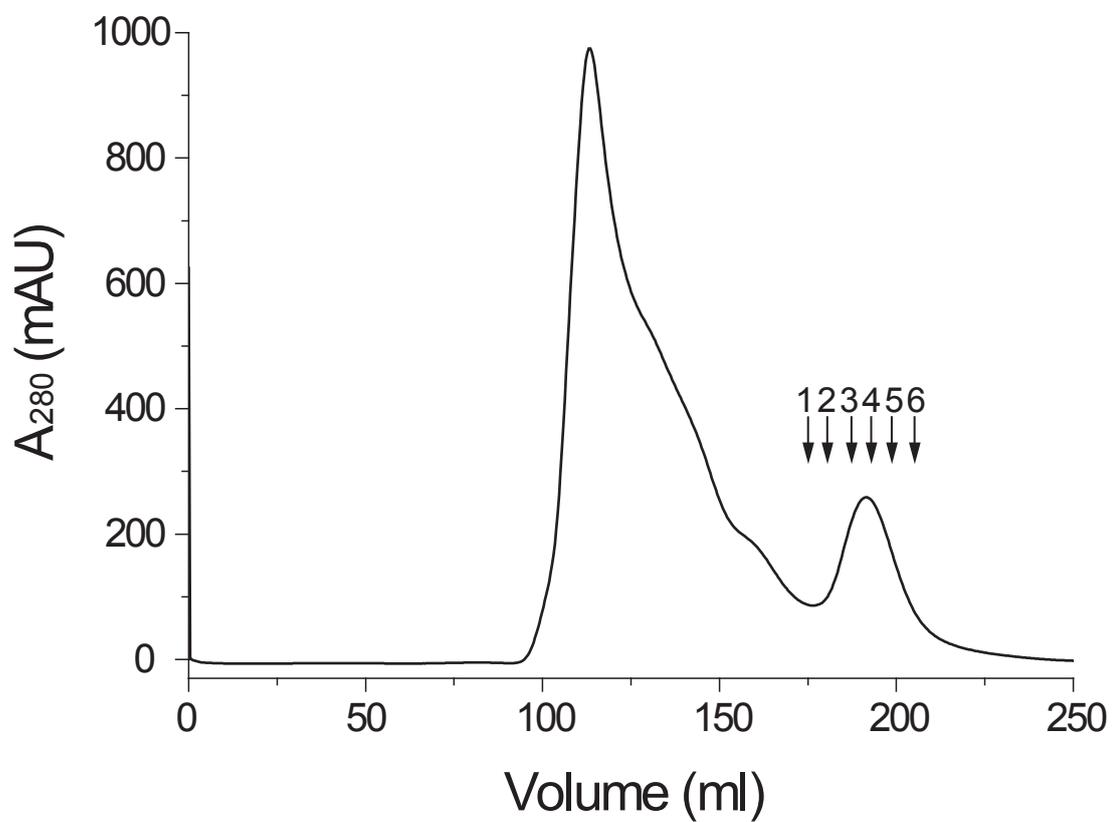


B



Supplemental Figure 5

A



B

