Widespread HC-10 Reactivity Across HLA Class I–Expressing Cell Types

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Abstract

Purpose: HC-10 recognizes a sequence- and conformation-dependent determinant of HLA class I associated with residue R62. This study evaluated whether HC-10 reactivity observed on HLA class I single antigen beads (SABs) is also present across primary human lymphocytes, HLA-defined cell lines, and endothelial cells under inflammatory conditions relevant to transplantation.

Methods: HC-10 reactivity was evaluated on LABScreen™ Single Antigen HLA Class I beads, PBMCs isolated from commercially sourced blood samples from 10 individual donors, homozygous EBV-transformed B-cell lines, and recombinant cell lines expressing individual HLA-I alleles. HUVECs were stimulated with IFNγ and/or TNFα to model endothelial inflammation. HLA-I expression and HC-10 reactivity were assessed by flow cytometry, and endothelial activation was evaluated using inflammatory markers.

Results: HC-10 reactivity was detected on HLA-I SABs and was associated with the R62-containing determinant. HC-10-reactive HLA-I was also detected on primary CD3$^+$ T cells and CD19$^+$ B cells, EBV-transformed B-cell lines, and recombinant single-allele HLA-I cell lines. The association between HC-10 reactivity and R62 was also observed in the single-allele cellular model. In endothelial cells, inflammatory stimulation increased HLA-I expression and HC-10 reactivity. Collectively, HC-10 reactivity was observed across bead-based and cellular systems, including primary and inflammation-responsive cell types.

Introduction

Single antigen bead (SAB) assays enable sensitive detection and characterization of HLA-specific antibodies. HC-10 is a monoclonal antibody that preferentially recognizes open/free heavy-chain HLA class I conformations. **HC-10 recognition is sequence dependent and strongly influenced by arginine at position 62 (R62) within the HLA-I α1 domain near the peptide-binding groove.**

HC-10 reactivity on HLA class I SABs has been associated with open or free heavy-chain HLA conformations and may be interpreted as evidence of non-physiologic or denatured HLA. However, HC-10-reactive HLA-I molecules have also been reported on the surface of intact cells, indicating that HC-10 reactivity is not necessarily restricted to artificially altered HLA.

Determining whether HC-10 reactivity is unique to SAB-bound HLA or also occurs in physiological cellular contexts is therefore important for interpreting HC-10 reactivity on HLA-I SABs.

Materials and Methods

Sample Preparation

LABScreen™ Single Antigen HLA Class I beads, primary human lymphocytes, homozygous EBV-transformed B-cell lines, recombinant single-allele HLA-I cell lines, and human umbilical vein endothelial cells (HUVECs) were evaluated.

PBMCs were isolated in-house from commercially sourced blood samples from 10 individual donors. CD3$^+$ T cells and CD19$^+$ B cells were evaluated within the PBMC population.

HUVECs were stimulated for 24 h with IFNγ (50 ng/mL), TNFα (10 ng/mL), or IFNγ + TNFα.

Test Methods

LABScreen™ Single Antigen HLA Class I beads (Cat:LS1A04, Thermo Fisher Scientific) were stained with W6/32 (Cat: MA5-11723 , Thermo Fisher Scientific) and HC-10 (Cat: MUB2037P, Thermo Fisher Scientific) monoclonal antibodies, and median fluorescence intensity (MFI) was determined for individual HLA-A, HLA-B, and HLA-C antigens.

Primary lymphocytes, EBV-transformed B cells, recombinant HLA-I cell lines, and HUVECs were analyzed by flow cytometry for surface W6/32 and HC-10 reactivity.

For PBMC analysis, HC-10 reactivity was quantified as the percentage of HC-10$^+$/W6/32$^+$ cells within CD3$^+$ T-cell and CD19$^+$ B-cell populations. HC-10 positivity was defined using the HC-10 fluorescence-minus-one (FMO) control.

Endothelial activation was evaluated by the expression of VCAM-1, ICAM-1, and E-selectin and secretion of IL-8 and CCL2.

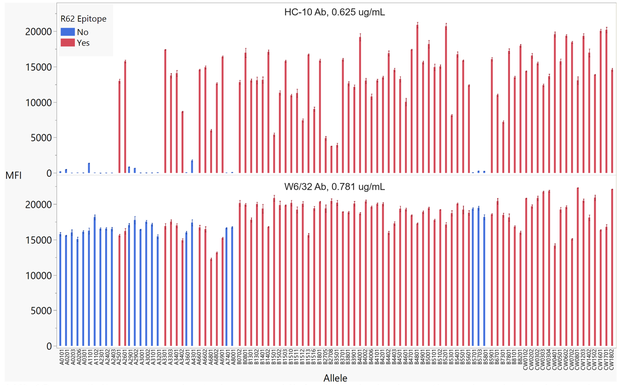
Data Analysis

Flow cytometry data were analyzed using FlowJo™ software. HC-10 and W6/32 reactivity were evaluated by population frequency and/or fluorescence intensity as appropriate for each experimental system.

For HUVEC experiments, MFI values were normalized to the average unstimulated control. Data are shown as mean ± SEM (n = 3), with statistical comparisons performed by one-way ANOVA followed by Tukey's multiple comparisons test.

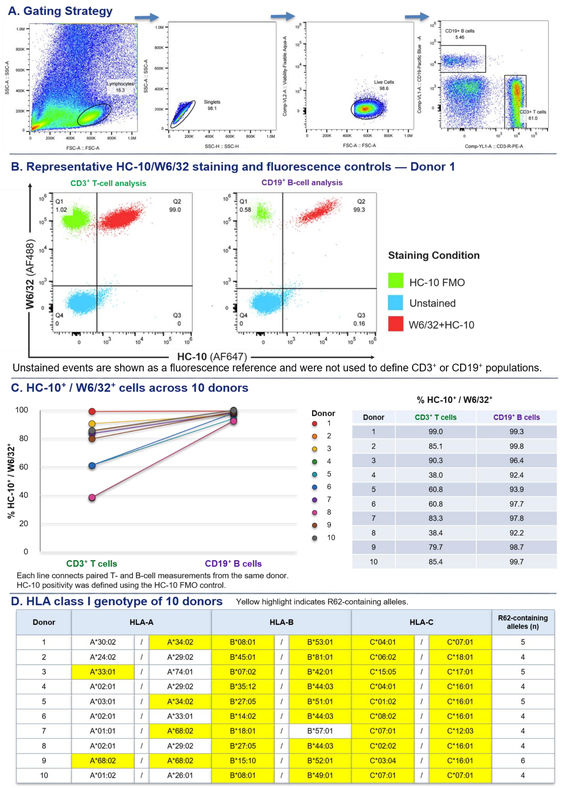
Results

Figure 1. HC-10 reactivity on LABScreen™ Single Antigen HLA Class I beads is associated with the R62 epitope



HC-10 reactivity was evaluated across HLA class I single antigen beads to determine whether antibody binding follows an allele-specific pattern. HC-10 reactivity was strongly associated with HLA-I alleles containing R62, whereas W6/32 showed broad reactivity across the bead panel, demonstrating sequence-dependent HC-10 recognition.

Figure 2. HC-10-reactive HLA class I is detected on primary human T and B lymphocytes



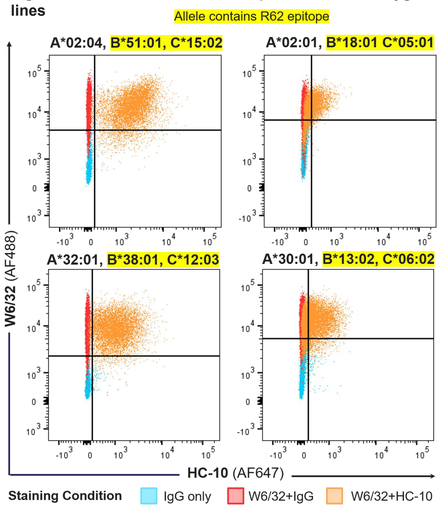
Donor	CD3⁺ T cells	CD19⁺ B cells
1	99.0	99.3
2	85.1	99.8
3	90.3	96.4
4	38.0	92.4
5	60.8	93.9
6	60.8	97.7
7	83.3	97.8
8	38.4	92.2
9	79.7	98.7
10	85.4	99.7

D. HLA class I genotype of 10 donors Yellow highlight indicates R62-containing alleles.

Donor	HLA-A		HLA-B		HLA-C		R62-containing alleles (n)
1	A*30:02	A*34:02	B*08:01	B*53:01	C*04:01	C*07:01	5
2	A*24:02	A*29:02	B*45:01	B*81:01	C*06:02	C*18:01	4
3	A*33:01	A*74:01	B*07:02	B*42:01	C*15:05	C*17:01	5
4	A*02:01	A*29:02	B*35:12	B*44:03	C*04:01	C*16:01	4
5	A*03:01	A*34:02	B*27:05	B*51:01	C*01:02	C*16:01	5
6	A*02:01	A*33:01	B*14:02	B*44:03	C*08:02	C*16:01	4
7	A*01:01	A*68:02	B*18:01	B*57:01	C*07:01	C*12:03	4
8	A*02:01	A*29:02	B*27:05	B*44:03	C*02:02	C*16:01	4
9	A*68:02	A*68:02	B*15:10	B*52:01	C*03:04	C*16:01	6
10	A*01:02	A*26:01	B*08:01	B*49:01	C*07:01	C*07:01	4

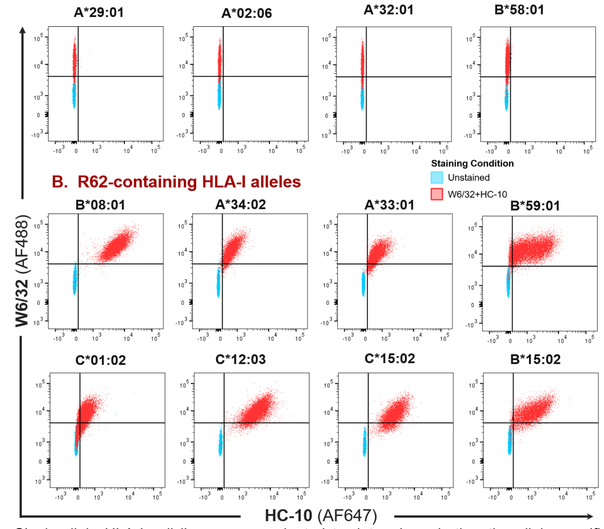
Primary human lymphocytes were evaluated to determine whether HC-10 reactivity observed on SABs is also present on naturally expressed cell-surface HLA-I. Across 10 individual donors, HC-10-reactive HLA-I was detected on both CD3$^+$ T cells and CD19$^+$ B cells, with consistently high reactivity on B cells and greater inter-donor variability among T cells.

Figure 3. HC-10-reactive HLA-I is present on homozygous EBV-transformed B-cell lines



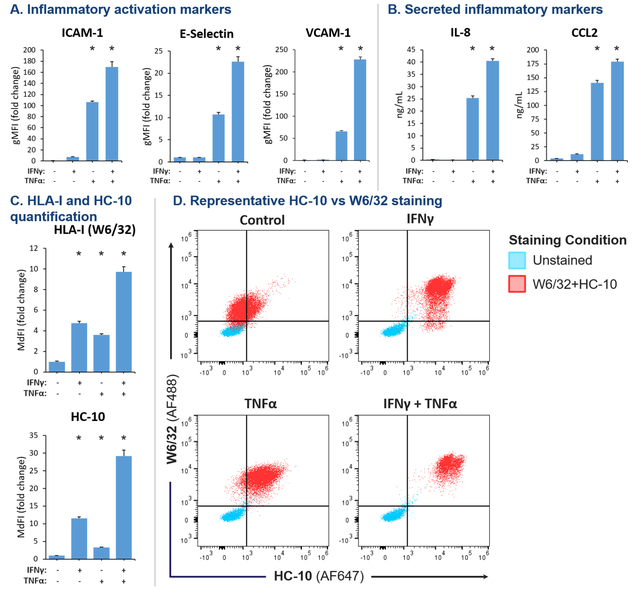
Four homozygous EBV-transformed B-cell lines expressing distinct HLA-I genotypes were stained with W6/32 and HC-10 and analyzed by flow cytometry. R62-containing alleles are highlighted. Overlay plots show IgG-only, W6/32 + IgG, and W6/32 + HC-10 staining conditions. HC-10-reactive HLA-I was detected on homozygous EBV-transformed B-cell lines, demonstrating that HC-10 reactivity occurs on naturally expressed cell-surface HLA-I and is not restricted to SAB-bound HLA.

Figure 4. HC-10 reactivity on recombinant single-allele HLA-I cell lines is associated with the R62-containing determinant



Single-allele HLA-I cell lines were evaluated to determine whether the allele-specific HC-10 pattern observed on SABs could also be reproduced on viable cells. HC-10 reactivity was preferentially observed among cell lines expressing R62-containing HLA-I alleles, whereas R62-negative alleles showed minimal reactivity. Allele-specific HC-10 reactivity associated with R62 was reproduced on viable single-allele cells, paralleling the sequence-dependent pattern observed on HLA-I SABs.

Figure 5. Inflammatory stimulation increases HLA-I expression and HC-10 reactivity in endothelial cells



Endothelial cells were evaluated to determine whether HC-10-reactive HLA-I is modulated under inflammatory conditions relevant to transplantation. Cytokine stimulation induced endothelial activation, as demonstrated by increased cell-surface inflammatory markers and secretion of inflammatory mediators. This activation was accompanied by increased HLA-I expression and HC-10 reactivity, with the greatest increases observed under combined IFNγ and TNFα stimulation. Inflammatory activation increases both HLA-I expression and HC-10 reactivity on endothelial cells, demonstrating that HC-10-reactive HLA-I is dynamically regulated in a physiologically relevant cellular context.

Conclusions

- **HC-10 reactivity is not unique to HLA class I single antigen beads.** HC-10-reactive HLA-I was also detected on primary T and B lymphocytes, EBV-transformed B-cell lines, recombinant single-allele cells, and endothelial cells.
- **HC-10 recognition is allele dependent and strongly associated with the R62-containing determinant.** This relationship was observed on both HLA-I SABs and viable recombinant single-allele cells.
- **HC-10-reactive HLA-I occurs in physiologically relevant cellular contexts.** Inflammatory stimulation increased HLA-I expression and HC-10 reactivity on endothelial cells, a key target of alloimmune responses during transplantation.
- **Collectively, these findings demonstrate that HC-10 positivity alone is insufficient to classify SAB-bound HLA as non-physiologic or denatured.** HC-10 reactivity should instead be interpreted in the context of HLA sequence, conformational state, and biological environment.

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