Figures and figure supplements

Variations in HLA-B cell surface expression, half-life and extracellular antigen receptivity

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Figure 1. Expression variations among HLA-Bw6 alleles. Forty-three healthy donors (Figure 1—source data 1) with either heterozygosity for HLA-Bw4/Bw6 or homozygosity for HLA-Bw6 alleles were sorted into six groups based on their Bw6 alleles. ABC values were calculated by flow cytometry based on staining freshly isolated PBMCs with anti-Bw6 or W6/32 and normalizing the resulting geometric MFI values against beads with known amounts of Fc receptors. Averaged ABC values for each donor are shown, grouped by the donor’s HLA-Bw6 alleles and lymphocyte subset analyzed (B cells (top row), CD4+ T cells (second row), CD8+ T cells (third row), and NK cells (last row)). For homozygous donors, 50% of the derived ABC values are plotted. Bw6 ABC values alone (column 1), W6/32 ABC values alone, (column 2) and the Bw6/W6/32 ABC ratios (column 3) are shown. The number of replicate measurements for each donor and standard errors of the mean are shown in Figure 1—source data 1. Statistically significant differences between alleles were analyzed by one-way ANOVA analysis for each cell type. Each dot represents averaged Bw6, W6/32, or Bw6/W6/32 ABC measurements (n > 3) from a single donor. p *<0.05; **<0.01; ***<0.001; ****<0.0001. This figure has five supplementary figures and one source data table.

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Figure 1—figure supplement 1. Peptide-binding motifs of several HLA-Bw6 allotypes relevant to this study. Peptides were analyzed using seq2logo: http://www.cbs.dtu.dk/biotools/Seq2Logo/ (Thomsen and Nielsen, 2012). Peptides for the boxed allele (left panel) were derived from a published dataset based on the immunoaffinity method (Abelin et al., 2017) and others were obtained from a published dataset based on acid elution of cells and epitope predictions (Pearson et al., 2016).

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Validations of anti-Bw6. Top panel: Anti-Bw6 (One Lambda BiH0038) was assessed for binding to different HLA class I conjugated to Luminex beads (Class I-LS1A04NC. LABScreen, One Lambda Inc.). Signals are plotted as ratios relative to those obtained with W6/32, a control antibody.

Figure 1—figure supplement 2 continued on next page
Figure 1—figure supplement 2 continued

pan HLA class I antibody. Bottom panels: Relative binding of anti-Bw6 to HLA-B allotypes relevant to this study. Binding was similar across the Bw6 allotypes for which expression and stability measurements are reported here. Data are based on two independent binding measurements.

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Figure 1—figure supplement 3. Sequences of HLA-B and HLA-C alleles with a Bw6 motif. Relevant HLA-B and HLA-C alleles are aligned at the Bw4/6 region.

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Figure 1—figure supplement 4. Representative RT-PCR measurements of HLA-B and total class I RNA levels for donors expressing indicated Bw6 alleles. Three technical replicates with the same cDNA samples were conducted.
Figure 1—figure supplement 4 continued

and a one-way ANOVA analysis of the 2^ΔΔt values was used to determine significance. Each dot represents the mean 2^ΔΔt values across three technical replicates from isolated cells from individual donors expressing the indicated HLA-B allele. Neither the HLA-B specific primer (top panels) nor the total Class I primer (lower panels) showed significant differences in mRNA levels. There was a trend of higher expression in CD8^+ T cells but it did not rise to significance. This result was consistent across two biological replicates comparing alleles that showed differences in ABC measurements.

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Figure 1—figure supplement 5. HLA-B mRNA expression within four lymphocyte populations in donors from Africa and Thailand. Total HLA-B expression of each individual was plotted for the HLA-B allele of interest and averaged expression was compared between alleles for each cell subset. There were no significant differences observed between HLA-B allele mRNA expression in CD4 T cells, B cells and NK cells in all donors consisting of samples from Africa and Thailand. A significant association was observed in the CD8 T cells.

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Figure 2. Cell surface stabilities of HLA-Bw6 allotypes are allele-dependent. Left column: Representative cell surface stability measurements of Bw6 epitopes on freshly isolated lymphocytes derived from Bw4/Bw6 heterozygous donors expressing HLA-B*08:01, HLA-B*35:01 or HLA-B*07:02 as the
Figure 2 continued

Bw6 allotype. Right column: Bw6 half-lives from Figure 2—source data 1 are grouped by Bw6 allele. Each data point represents data derived from an individual donor, with the open data points representing donors shown in the left panel. Mean half-life values are shown for each donor, measured using freshly isolated cells from at least two independent blood collections for each donor. The number of replicate measurements for each donor and standard errors of the mean are shown in Figure 2—source data 1. Statistical significance is based on one-way ANOVA analysis. p *<0.05, **<0.01, ***<0.001, and ****<0.0001 This figure has one source data table.

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Figure 3. Altered patterns of HLA-Bw6 surface expression and stability in monocytes compared with lymphocytes. A and B: Blood donations were again obtained from a subset of donors represented in the Figure 1 measurements. Averaged ABC values measured with anti-Bw6 (A) or W6/32 (B) for each donor are shown, grouped by the donor’s HLA-Bw6 alleles and cell subsets. C: For each donor represented in A and B, Bw6 ABC values in lymphocytes are normalized relative to the monocyte values from the same donor, and grouped by the donor’s HLA-Bw6 alleles and cell subsets. Averaged ABC values and data replicates obtained for plots in A-C are shown in Figure 3—source data 1. D: Cell surface stability measurements (obtained as described in Figure 2) of CD4⁺ and CD8⁺ T cells in comparison to monocytes. E: Cell surface stability measurements in monocytes of indicated HLA-Bw6 allotype. Half-life values and data replicates obtained for the plots in D and E are shown as Figure 3—source data 2. A-E: Each point represents data from a single donor. Statistical significance is based on one-way ANOVA analysis. p *<0.05, **<0.01, ***<0.001, and ****<0.0001.

This figure has two source data tables.

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Figure 4. HLA class I assembly differences between monocytes and lymphocytes. A: Flow cytometry experiments measuring W6/32-based staining of cell surface HLA class I (fixed PBMCs) expressed as a ratio relative to W6/32-based staining of total HLA class I (fixed and permeabilized PBMCs). Each point represents an individual donor measurement, and a total of 33 donor samples were tested. B: PBMCs were fixed and permeabilized, then stained with either anti-tapasin or W6/32 antibodies. The ratio of tapasin MFI relative to the W6/32 MFI was calculated for each cell type, then normalized to the corresponding monocyte ratios. Each point represents an individual donor measurement, and a total of 29 donor samples were tested. C and D: Summary statistics from two ImageStream experiments with three donors in monocytes (C) or CD4+ T cells (D). Bw6 and AP-1 co-localization, Bw6 and CRT co-localization, and Bw6 and LAMP-1 co-localization were quantified for donors 94 and 64. Only Bw6 and AP-1 co-localization was measured for donor 237. E and F: Representative monocyte (E) or CD4+ T cell (F) images for the experiments summarized in Panels C and D. This figure has five supplementary figures and one source data table.

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Tapasin expression. Intracellular tapasin expression measured by flow cytometry with the monoclonal PaSta-1 antibody, and the data were normalized to monocyte expression levels. Each point represents a single measurement on 33 donors. One-way ANOVA analysis was performed, with p ****<0.0001.

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Figure 4—figure supplement 2. Gating strategy for imaging cytometry experiments. PBMCs were analyzed on the Amnis ImageStreamX imaging cytometer and gated for cell populations. These populations were then analyzed for colocalization of Bw6 with either AP-1, calreticulin (CRT), or LAMP-1. Gating strategies for monocytes and CD4^+ T cells are shown in panel R2. In this panel, CD3-Pacific Blue is plotted on the X axis, and CD8-Alexa Fluor 700 and CD14-Alexa Fluor 700 are plotted on the Y axis. CD4^+ T cells were identified by gating on the CD3^+, CD8^-cells (gate R3), and monocytes were identified by gating CD3^-, CD14^+ cells (gate R4). These populations were then gated on cells that were double positive for the two co-localization markers of interest, and these double positive cells were analyzed for Bright Detail Similarity (BDS) in panels R5 and R8. BDS is a quantification of the degree of overlap between two markers. Thus, cells with a high BDS score have a high degree of co-localization between the two markers analyzed. Panels R5 and R8 show the gates used to quantify cells with high, intermediate, and low co-localization.

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Figure 4—figure supplement 4. Representative image gallery for Donor 94 monocytes. PBMCs from donor 94 were analyzed by imaging cytometry for Bw6/AP-1 colocalization. The top panel represents gate R9—monocytes with high Bw6/AP-1 colocalization—while the bottom panel represents gate R12—monocytes with intermediate Bw6/AP-1 colocalization (defined as in Figure 4—figure supplement 2).

DOI: https://doi.org/10.7554/eLife.34961.019
Representative image gallery for Donor 237 monocytes. PBMCs from donor 237 were analyzed by imaging cytometry for Bw6/AP-1 colocalization. The top panel represents gate R9—monocytes with high Bw6/AP-1 colocalization—while the bottom panel represents gate R12—monocytes with intermediate Bw6/AP-1 colocalization (defined as in Figure 4—figure supplement 2).

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Figure 5. Lymphocyte HLA-B*35:01 and HLA-B*07:02 are receptive to exogenous peptides. PBMCs were freshly isolated from healthy donors expressing one copy of the indicated HLA-B allele and incubated with 100 μM of specific or matched control peptides for each allotype for four hours.

Figure 5 continued on next page
Figure 5 continued

at 37°C. The cells were then stained with an antibody cocktail containing antibodies to differentiate lymphocyte subsets, as well as HC10, a monoclonal antibody that recognizes peptide-deficient HLA class I molecules. The data are shown for CD4+ and CD8+ T cells, B cells, NK cells, and monocytes. Data are representative of 1-2 separate measurements for each donor, with 3-5 donors per allele, as specified in Figure 5—source data 1. This figure has one source data table.

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Figure 6. Cell-derived Bw4 ABC values correlate with anti-Bw4 binding preferences (with the exception of B*51:01) and similar cell surface stabilities are measured for the indicated Bw4 allotypes. Columns 1 and 2: Lymphocyte ABC values for Bw4/Bw6 heterozygous donors expressing indicated Bw4 genotypes (and lacking cross-reactive HLA-A) were measured using anti-Bw4 and W6/32 (all donor information is specified in Figure 6—source data 1). Resulting Bw4 ABC data (column 1) or Bw4/W6/32 ABC ratios (column 2) are grouped for donors based on their Bw4 genotypes, and plotted against the corresponding Luminex Bw4/W6/32 signals obtained from Figure 6—figure supplement 1). Column 3: Averaged cell surface stability.
Figure 6 continued

measurements of Bw4 epitopes on freshly isolated lymphocytes derived from Bw4/Bw6 heterozygous donors expressing indicated Bw4 allotypes. Half-life statistical significance is based on one-way ANOVA analysis using data in Figure 6—source data 2. This figure has four supplementary figures and two source data tables.

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Specificity and relative binding propensity of anti-Bw4. Top panel: Anti-Bw4 (One Lambda BiH0007) was assessed for binding to different HLA class I conjugated to Luminex beads (Class I-LS1A04NC. LABScreen, One Lambda Inc.). Signals are plotted as ratios relative to Yarzabek et al. eLife 2018;7:e34961. DOI: https://doi.org/10.7554/eLife.34961

Figure 6—figure supplement 1. Specificity and relative binding propensity of anti-Bw4. Top panel: Anti-Bw4 (One Lambda BiH0007) was assessed for binding to different HLA class I conjugated to Luminex beads (Class I-LS1A04NC. LABScreen, One Lambda Inc.). Signals are plotted as ratios relative to Figure 6—figure supplement 1 continued on next page
those obtained with W6/32, a pan HLA class I antibody. Bottom panels: Relative binding of anti-Bw4 to HLA-B allotypes relevant to this study. Differences are measured in the binding of anti-Bw4 across the Bw4 allotypes for which expression and stability measurements are reported here. Data are based on two independent binding measurements.

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**Figure 6—figure supplement 2.** Sequences of HLA-B and HLA-A alleles with a Bw4 motif. Relevant HLA-B and HLA-A alleles are aligned at the Bw4/6 region.

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Figure 6—figure supplement 3. Peptide-binding motifs of several HLA-Bw4 allotypes relevant to this study. Peptides were analyzed using seq2logo: http://www.cbs.dtu.dk/biotools/Seq2Logo/ (Thomsen and Nielsen, 2012). Peptides for the boxed alleles (left panel) were derived from a published dataset based on the immunoaffinity method (Abelin et al., 2017) and peptides for the right panels were obtained from a published dataset based on acid elution of cells and epitope predictions (Pearson et al., 2016). Neither study included HLA-B*37:01, which belongs to the B44 supertype, and thus is expected to have a motif similar to B*44:02. B*13:02 was described in Pearson et al. (2016), but epitope prediction tools are not well-developed for this allotype. DOI: https://doi.org/10.7554/eLife.34961.027
Figure 6—figure supplement 4. Expression measurements of HLA-Bw4 alleles. Donors with heterozygosity for HLA-Bw4/Bw6 and lacking HLA-A allotypes cross-reactive with anti-Bw4 were sorted into six groups based on their Bw4 alleles. ABC values were calculated by flow cytometry based on staining freshly isolated PBMCs with anti-Bw4 and W6/32 and normalizing the resulting geometric MFI values against beads with known amounts of Fc receptors. Averaged ABC values for each donor are shown, grouped by the donor’s HLA-Bw4 alleles and lymphocyte subset analyzed (B cells (top row), CD4+ T cells (2nd row), CD8+ T cells (3rd row), and NK cells (last row)). Bw4 ABC values alone (left column), W6/32 ABC values alone, (middle column)