CD160 Is the Most Specific Co-inhibitory Molecule Associated With CD8 T Cell Functional Impairment

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Abstract

Background: Co-inhibitory molecules are commonly associated with T-cell dysfunction in the context of chronic viral infections such as HIV or HCV, however, their relative contribution in T-cell impairment remains unclear.
Methodology: In the present study, we evaluated the influence of expression of co-inhibitory molecules *i.e.* 2B4, PD-1 and CD160 on CD8 T-cell functions using the prototypic models of cleared (Flu) and persistent (EBV, CMV) virus infections.
Results: We hereby show that 2B4$^+$CD160$^+$PD-1$^+$ and 2B4$^+$CD160$^+$PD-1$^-$ CD8 T-cell populations are endowed with a reduced capacity to proliferate, produce IL-2 and IFN-γ, associated with a reduced capacity to express perforin, demonstrating that CD160$^+$ CD8 T cells are functionally impaired, independently of PD-1 expression. In addition, the blockade of CD160/CD160-ligand interaction restored CD8 T-cell proliferative capacity and the level of CD8 T-cell proliferative restoration directly correlated with the *ex vivo* proportion of CD160 expression, demonstrating that CD160 signaling negatively regulates TCR-mediated CD8 T-cell signaling. Finally, in contrast to PD-1, CD160 expression was not up-regulated upon T-cell activation or proliferation.
Conclusions: Taken together, this study demonstrates that CD160 is the most specific co-inhibitory molecule associated with CD8 T-cell dysfunctions.

Materials & Methods

Study group. Forty subjects were recruited in this study.
***Ex vivo* analyses of CD8 T cells**. Cryo-preserved PBMCs were stained with peptide-MHC class I multimer complexes and anti-CD3, CD8, CD4, PD-1, CD160 and 2B4 MAbs.
Virus-specific CD8 T-Cell proliferation capacity. Proliferation capacity is represented in proliferation index, defined as the ratio of the virus-specific CD8 T-cell proliferation capacity (percentage of CFSE low CD8 T cells) and the *ex vivo* frequency of virus-specific CD8 T cells (measured by MHC class I multimer staining).
Intracellular cytokine staining (ICS). PBMC were stimulated overnight in the presence of Golgiplug and anti-PD1, anti-CD160 and anti-2B4 mAbs. At the end of the stimulation period, cells were stained with anti-CD3, CD8, CD45RA, CCR7, IFN-γ and IL-2 MAbs.
Perforin and granzyme B expression assessment. PBMC were stained anti-CD3, CD8, PD-1, 2B4, CD160, CCR7, CD45RA, perforin and granzyme B MAbs.
Flow cytometry analyses. Cells were acquired on an LSRII SORP (4 lasers: 405, 488, 532 and 633 nm) and analyzed using FlowJo (version 8.8.2)
Statistical analyses. Statistical significance (*P* values) was obtained using one-way ANOVA (Kruskal-Wallis test) followed by Student's *t* test for multiple comparisons or a Spearman rank test for correlations.

Results

Figure 1. EBV and CMV-specific CD8 T cells express significantly higher levels of CD160 than Flu-specific CD8 T cells

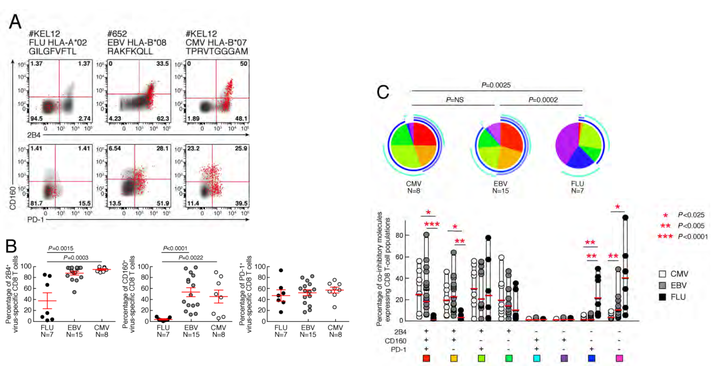


Figure 2. CD160 but not PD-1 and/or 2B4 expression negatively correlates with CD8 T-cell proliferative capacity

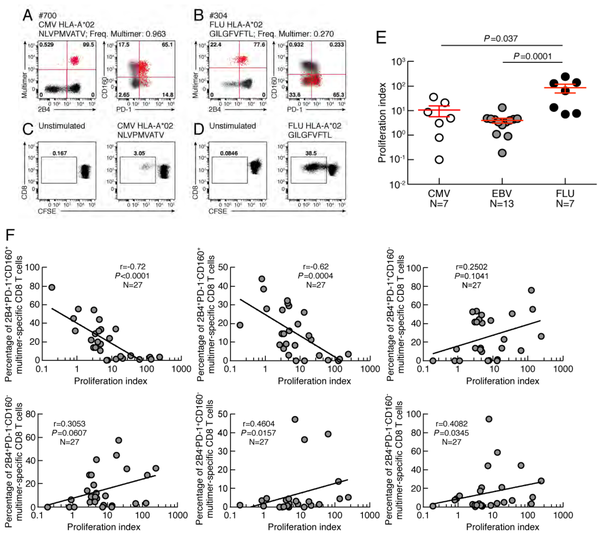


Figure 3. CD160 but not PD-1 expression is strongly associated with reduced IL-2 production

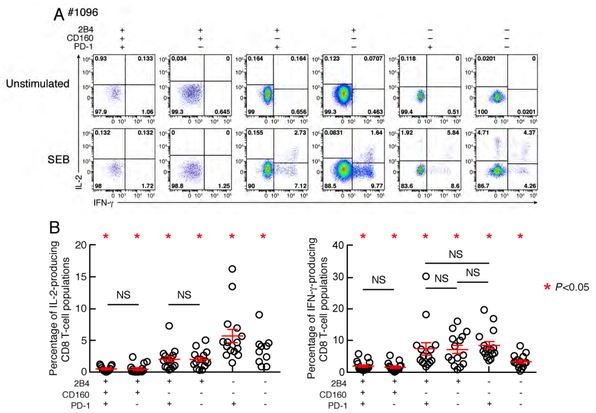


Figure 4. Specificity of Different Ad Serotype NAbs forming ICs in the Induction of TNF-α production by DCs

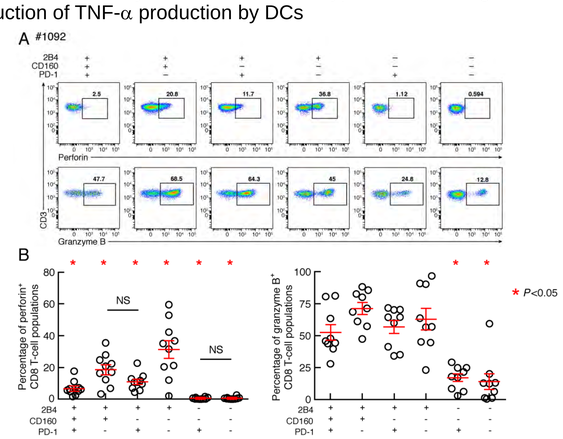


Figure 5. CD160 but not PD-1 expression is strongly associated with reduced perforin expression

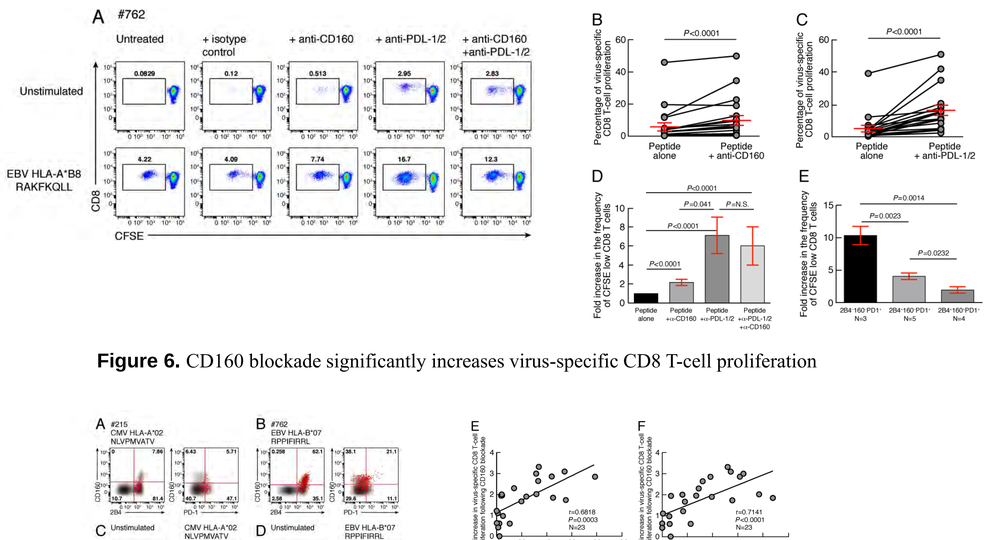


Figure 6. CD160 blockade significantly increases virus-specific CD8 T-cell proliferation

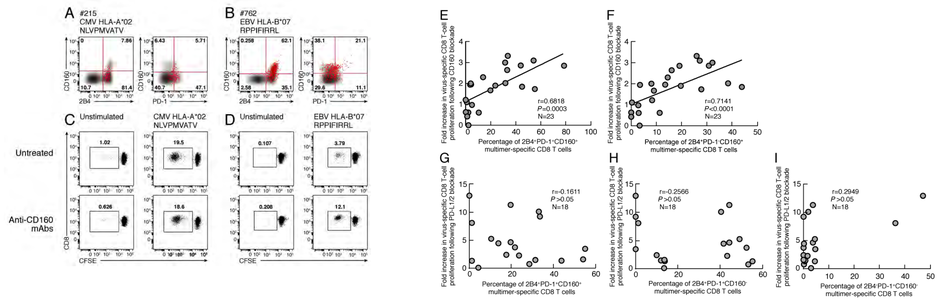
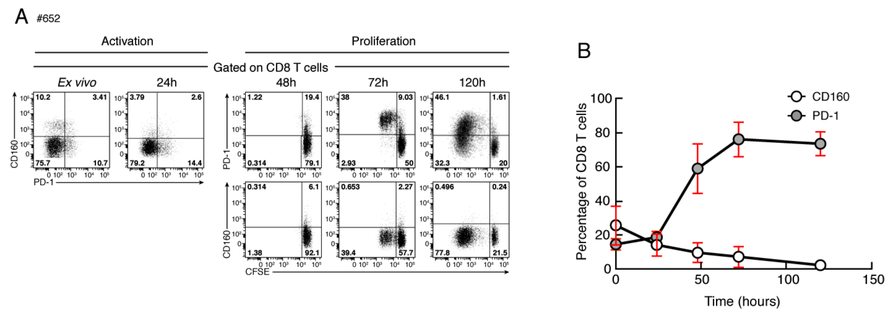


Figure 7. Restoration of CD8 T-cell proliferation by CD160/CD160-ligand blockade directly correlates with the level of the *ex vivo* CD160 expression



Conclusions

1. CD160-expressing CD8 T-cell populations are endowed with a reduced capacity to proliferate, to produce IL-2 and IFN-γ and are associated with a reduced capacity to express perforin regardless of PD-1 expression.
2. The 2B4$^-$CD160$^-$PD-1$^+$ CD8 T-cell population was significantly enriched in Flu-specific CD8 T cells and characterized by higher IL-2 production capacity suggesting that this CD8 T cell population is less functionally impaired than 2B4$^+$CD160$^-$PD-1$^+$ and 2B4$^+$CD160$^+$PD-1$^+$ CD8 T cell populations.
3. The blockade of CD160/CD160-ligand restored CD8 T cell proliferation capacity and the degree of restoration correlated with the *ex vivo* proportion of CD160 expression thus demonstrating that CD160 signaling negatively regulates TCR-mediated CD8 T cell signaling.
4. In contrast to PD-1, CD160 expression was not up-regulated upon T-cell activation or proliferation.