Isolation, purification and crystallization of a novel bidirectional NK activating ligand CD160 in complex with herpesvirus entry mediator HVEM

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Many pathogens have evolved countermeasures to avoid detection and clearance by natural killer (NK) cells [1,2], however, regulatory mechanisms limiting cytokine activation of NK cells that reduce non-specific tissue damage remain poorly defined. CD160 is a 27 kDa glycoprotein which was initially identified with the monoclonal antibody BY55 [2]. Its expression is tightly associated with peripheral blood NK cells and CD8 T lymphocytes with cytolytic effector activity [2,3,4]. The cDNA sequence of CD160 predicts a cysteine-rich, glycosylphosphatidylinositol-(GPI)-anchored protein of 181 amino acids with a single Ig-like domain weakly homologous to KIR2DL4 molecule [Fig.1C]. It was found that TNF receptor herpesvirus entry mediator (HVEM) preferentially engages CD160 trimer to costimulate activation, while a viral ortholog of HVEM specifically binds to B and T lymphocyte attenuator (BTLA) to suppress this signaling. Thus, regulation of CD160 bidirectional binding may represent a common mechanism of immune regulation targeted by multiple pathogens, which by extension is a potential target for therapeutic manipulation. We have found that CD160 is expressed at the cell surface as a tightly disulfide-linked multimer. The homology model of CD160 antigen domain [Fig.1B] shows cysteine-rich region that was found to be responsible for CD160 tight-timer formation even under reduced conditions. CD160 trimer forms stable complex with HVEM, while monomeric form refused to binds its cognate ligand. We have attempted crystallization of HVEM and CD160 molecules in singles, as well as in complex. Protein crystals shown in Fig.1A were grown by using PEG ion and Morpheus crystal screens and successfully tested for X-ray diffraction.

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