

Novel Mechanism of Extracellular Antigen Cross-Presentation

September 16, 2013

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The ability of the immune system to recognize and control tumor cells and pathogens depends on the processing and presentation of antigens. Major histocompatibility complexes (MHC) present short protein fragments derived from tumors or pathogens on the surface of cells to activate T-cells which in turn target tumor or infected cells for elimination. All cell types express MHC class I (MHC-I) and can present processed intracellular antigens to stimulate cytotoxic CD8+ T-cell activation. Professional antigen-presenting cells (APCs), including dendritic cells, express MHC class I and class II and efficiently process both extracellular and intracellular antigens to activate CD8+ cytotoxic T-cells and CD4+ helper T-cells. Both viruses and tumor cells have evolved immune evasion strategies that prevent MHC-I peptide presentation directly from affected cells. In response, APCs have evolved mechanisms to efficiently uptake antigens released from infected or tumor cells and cross-present these antigens to CD8+ T-cells via MHC-I.

A new study by postdoctoral fellow Dr Jodie Goodridge and colleagues in the laboratory of Dr. Dan Geraghty (Clinical Research Division) have uncovered a novel mechanism of how non-professional APCs can load extracellular antigens onto MHC-I and activate an immune response. According to Dr. Geraghty this "model links an individual's host immune genetics – expressed from person to person in substantially variable structural forms – to any particular tumor or pathogen proteome."

Normal presentation of endogenous antigens and cross-presentation of exogenous antigens requires that proteins are degraded to small 8-10 amino acid peptides by the proteasome. These peptides are then loaded onto MHC-I in the endoplasmic reticulum by the proteins TAP and tapasin, and then delivered to the cell surface. MHC-I bound to peptide is then recognized by T-cell receptors (TCRs) on cytotoxic T-cells. In addition, upon activation, most lymphoid or myeloid cell types upregulate the non-classical MHC class I HLA-F to the cell surface coincidentally with MHC-I in an open conformation (MHC-I OC) without peptide (Lee *et al.*, 2010). A previous study by Goodridge *et al.* demonstrated that HLA-F is also expressed peptide free as an open conformer and binds MHC-I OC on the cell surface of activated lymphocytes and monocytes (Goodridge *et al.*, 2010).

In the current study, the researchers asked if HLA-F helped load exogenous antigens to MHC-I for cross-presentation from activated lymphocytes and monocytes, both non-professional APCs that express HLA-F upon activation. Denatured viral proteins in both native denatured forms or as partial forms synthesized as polypeptides of 40-50 amino acid length, with known peptide epitopes that bind to MHC-I, were added exogenously to B-cells expressing HLA-F and MHC-I OC. HLA-F cell surface expression decreased as detected by flow cytometry, suggesting internalization of the complex once antigen bound to MHC-I. Using biochemical fractionation of cells, lysosomal inhibitors, and microscopic colocalization studies, the researchers traced the internalization of the complex from early endosomes into lysosomes where HLA-F and MHC-I dissociate and the exogenous antigens are further degraded into smaller peptides that load onto MHC-I. Importantly, the binding and internalization of the complex required denatured proteins with specific epitopes to bind MHC-I, as determined by amino acid substitutions in the peptide epitope region.

The researchers then examined if the activated B-cells could cross-present viral or tumor antigens to cytotoxic T-cells. Indeed, exogenously added antigen activated cytotoxic T-cells to specifically lyse the B-cells. Presentation of antigens and activation of T-cells was blocked with brefeldin A, a drug that disrupts the Golgi compartment, confirming direct processing of longer antigens to short peptide epitopes through internalization. Interference with the surface expression of HLA-F and MHC-I OC with short hairpin RNA constructs blocked the processing and cross-presentation of exogenous viral antigens to activate T-cells. Importantly, this pathway did not require the proteins TAP or tapasin as required for cross-presentation in most professional APCs. This same pathway was evident in activated cytotoxic T-cells that also express HLA-F, suggesting they can self-stimulate and cross-present antigens in inflammatory conditions.

The researchers suggest that HLA-F may stabilize MHC-I OC to and from the cell surface (see figure). The process requires denatured proteins, which could be released after degradation by proteases from apoptotic or cytolized cells in inflammatory conditions that also increase the expression of HLA-F on activated lymphocytes and monocytes. "This novel pathway suggests a simplified, precision approach that could be applied immediately for immunization against tumor antigens or pathogens," remarks Dr. Geraghty. "Currently, we are attempting to define the optima for these interactions, the 'rules of engagement' if you will, toward a new approach for vaccine development that can be broadly applied to cancer and infectious disease."

[Goodridge JP, Lee N, Burian A, Pyo CW, Tykodi SS, Warren EH, Yee C, Riddell SR, Geraghty DE.](#) 2013. HLA-F and MHC-I Open Conformers Cooperate in a MHC-I Antigen Cross-Presentation Pathway. *Journal of Immunology* 191:1567-77.

Also see: [Lee, N., Ishitani, A., and Geraghty, D.E.](#) 2010. HLA-F is a surface marker on activated lymphocytes. *Eur J Immunol.* 40, 2308-2318.

[Goodridge JP, Burian A, Lee N, Geraghty DE.](#) 2010. HLA-F complex without peptide binds to MHC class I protein in the open conformer form. *Journal of Immunology* 184:6199-208.

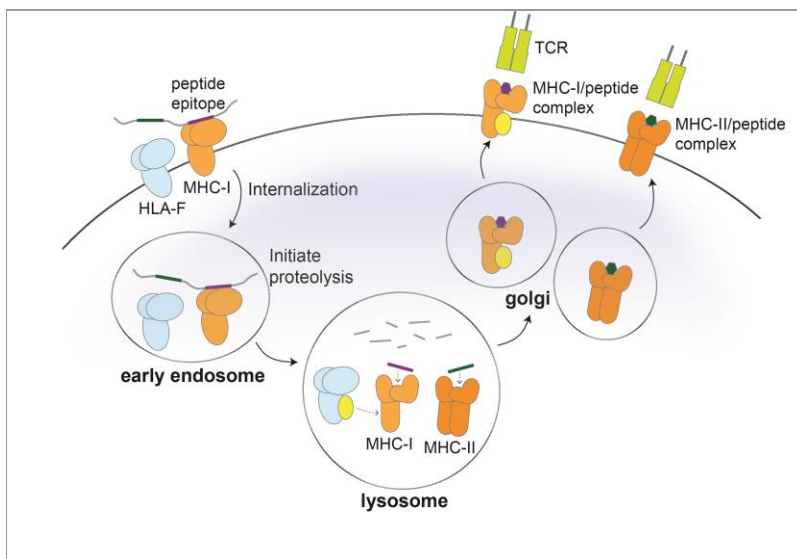


Image provided by Dan Geraghty

Proposed model of HLA-F function. HLA-F binds and stabilizes open conformers of MHC-I on the cell surface and helps load exogenous antigens for cross-presentation to activate T-cells.