Role of Nedd4 E3 Ligases in the Downregulation of CD1d and the Inhibition of NKT Cell Function

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Abstract

The CD1d protein presents lipid antigens to T cells. A subset of T cells, natural killer T (NKT) cells, is then activated and in response bridges the innate and adaptive immune arms because they can mass produce cytokines upon activation. With the collaboration of these two systems, the body can better suppress pathogenic and cancer cells and also influence diverse immune responses. However, many viruses have developed evasion methods to avoid the antigen presenting CD1d. Through mice models, the Yuan lab began investigation on the effect of Herpes Simplex Virus 1 (HSV 1) on the NKT/CD1d cell system. A newly discovered gene, UL56 has been found to efficiently downregulate the expression of CD1d and inhibit the function of NKT cells. UL56 has been hypothesized to do this through the Nedd4 family of E3 ligases, or ubiquitin ligases. Through lab techniques such as mammalian cell culture, transfection, western blotting, and flow cytometry, I set out to determine if UL56 recruits Nedd4 to degrade CD1d and suppress function of NKT cells in the immune system.

Background

UL56 is a viral protein encoded by members of the herpesvirus family. The UL56 protein is a component of the viral tegument, which is the layer between the viral capsid and the envelope in herpesviruses. Tegument proteins are essential for replication, viral assembly and egress from infected cells.

Nedd4 (Neural precursor cell Expressed Developmentally Down-regulated 4) is a family of E3 ubiquitin ligases, which are enzymes responsible for transferring ubiquitin molecules to target proteins, marking them for degradation by the proteasome or altering their function and localization within the cell. The Nedd4 family of E3 ligases plays a crucial role in various cellular processes, including protein turnover, endocytosis, signal transduction, and regulation of membrane proteins.

CD1d and NKT Cell Immune Relationship

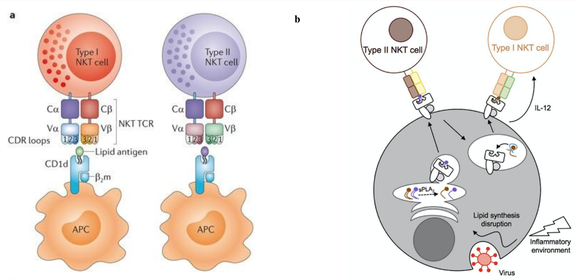


Figure 1. Recognition of CD1d restricted antigens by natural killer T cells
Figure 2. CD1d interactions with type 1 and 2 NKT cells

Upon recognition of the CD1d-antigen lipid complex, NKT cells are activated and rapidly release a variety of cytokines, such as interferon-gamma (IFN-γ) and interleukin-4 (IL-4). These cytokines modulate the immune response and influence the behavior of other immune cells.

In immunomodulation, the interaction between CD1d and NKT cells has been implicated in various diseases, including infections, cancer, autoimmunity, and allergy. NKT cells can promote inflammation and enhance antiviral responses, but they can also regulate immune tolerance and prevent autoimmune responses.

UL56 Expression Leads to CD1d Degradation

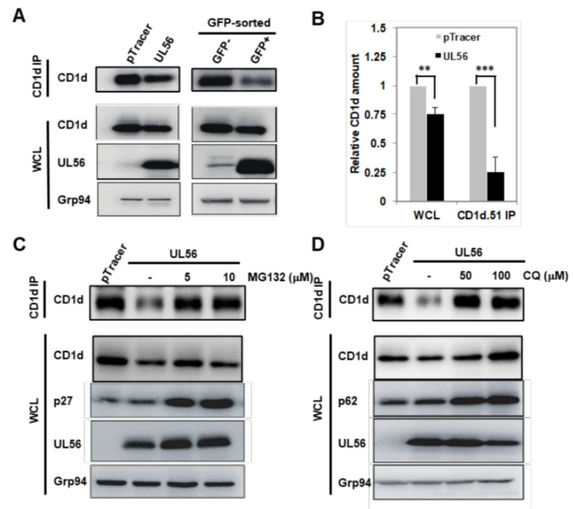


Figure 3. Presence of UL56 Corresponds to Decrease in CD1d

I transfected 293T.CD1d cells with either pTracer or pcDNA3 constructs with the UL56 protein. They were either untreated (A, B) or treated using MG132 or chloroquine at the indicated concentrations (C, D). Cell lysates were subject to immunoprecipitation with anti-CD1d antibodies, CD1d-51.1 and analyzed by western blotting with anti-CD1d antibody, CD1d-D5.

UL56 and Nedd4 Collaborate to Reduce CD1d

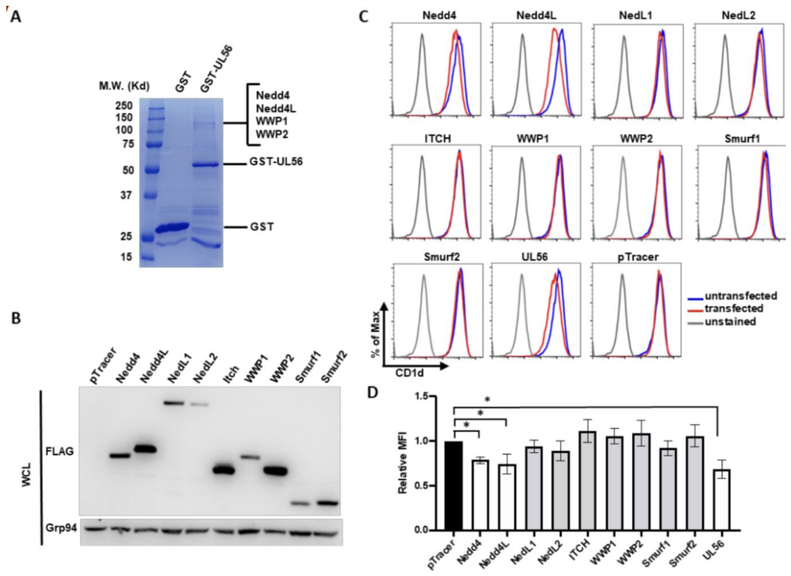
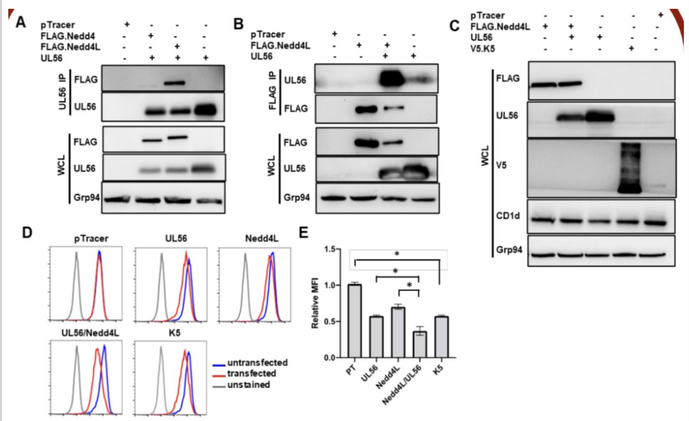


Figure 4. UL56 synergy with Nedd4 may suppress CD1d expression.

(A). Cellular proteins associated with UL56 were purified from cell lysates of transfected 293T.CD1d cells expressing GST-fused UL56 (1-215) by GST-pulldown. The high molecular weight bands were subjected to mass spectrometry to identify the co-purified proteins. Cells expressing GST protein were used as a control. (B-D). Individual members of FLAG-tagged Nedd4-family E3 ubiquitin ligases were co-transfected with pTracer plasmid in 293T.CD1d cells. The transfected cells were subjected to western blotting (B) or stained with the anti-CD1d antibody, CD1d-51.1 and cell surface CD1d expression was analyzed by flow cytometry (C, D). The transfection experiments were repeated three times and results were summarized in (D). Among other ubiquitin ligase families such as ITCH or Smurf2, Nedd4L and Ul56 clearly express the lowest amount of CD1d. This solidifies the assumption that UL56 binds to Nedd4L in order to discourage CD1d antigen presentation.

Nedd4L as culprit ubiquitin in deregulating CD1d/NKT



Through western blotting and co-immunoprecipitation, we deduced that Nedd4L, a ubiquitin ligase enzyme involved in the regulation of protein stability and turnover, was the chief ligase in downregulating CD1d. Nedd4L is the major Nedd4-family ubiquitin ligase to cooperate with UL56 to suppress CD1d expression. Nedd4L also plays a crucial role in the regulation of various ion channels and transporters in the cell membrane. By ubiquitinating these membrane proteins, Nedd4L can control their surface expression and activity, thus modulating cellular processes like ion transport and cellular excitability.

(A, B). 293T.CD1d cells were transfected with plasmids expressing UL56 alone or together with FLAG-tagged Nedd4L and subjected to co-immunoprecipitation with anti-UL56 antibodies or anti-FLAG antibodies. The immunoprecipitates were western blotted with anti-FLAG or anti-UL56 antibodies, respectively. (CD). 293T.CD1d cells were transfected to express UL56 and Nedd4L alone, UL56 plus Nedd4L, or KSHV K5 proteins. They were then subjected to western blotting (C) or cell surface staining for CD1d expression (D).

Summary

- UL56 is involved in a decrease in CD1d levels, which results in a stasis of NKT cell activity.
- UL56 recruits the Nedd4 family of E3 ligases through ubiquitination cascades and the proteasome and lysosome pathways.
- Among the E3 ubiquitin ligase family, Nedd4L is most efficient in degrading CD1d.
- HSV-1 requires UL56 in order to evade NKT cell/CD1d function.

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