STRUCTURAL BASIS FOR LAG3 ENGAGEMENT OF IMMUNOMODULATORY LIGANDS AND ANTIBODIES

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Background Lymphocyte activation gene 3 (LAG3) was recently validated as a target for next-generation immune checkpoint inhibitors. However, unlike PD-1 and CTLA-4, we have a poor molecular-level understanding of the LAG3 immunosuppression mechanism. We determined the structure of the LAG3 ectodomain to gain insight into its architecture and assembly on the surface of T cells. Subsequent functional studies identified key interfaces that mediate LAG3 engagement of antagonist antibodies and its cellular ligands Fibrinogen-like 1 (FGL1) and MHC class II.

Methods To facilitate structural studies of unstable LAG3 proteins, we used yeast display to evolve a human LAG3 variant with improved biochemical properties. This variant (named LAG3*) contains a single conservative M171I mutation and had increased expression yield, stability, and ligand-binding affinity compared to WT LAG3. The LAG3* protein was co-crystallized with an antagonist antibody fragment and the structure was determined to 3.7-angstrom resolution. Mutational mapping identified the binding sites of FGL1 and multiple antagonist antibodies on the LAG3 protein. We also used x-ray crystallography to map the LAG3-binding site of the FGL1 protein, and we used confocal microscopy to visualize LAG3-FGL1 complex formation on the surface of Jurkat T cells.

Results The structure of LAG3* revealed an elongated “X-shaped” architecture and a dimer interface formed by domain 2 (D2) of the LAG3 extracellular domain (ECD). A potent neutralizing antibody blocked LAG3 interactions with both FGL1 and MHCII and bound to a flexible “loop 2” region within LAG3 domain 1 (D1). This loop is distinct from the “loop 1” region previously implicated in MHCII binding and suggests that dual-ligand blockade can be achieved by targeting the loop 2 epitope. Structural modeling and microscopy studies revealed that FGL1 binding to LAG3 induces clustering on the cell surface, which may be important for disrupting proper immune synapse formation.

Conclusions Our structural studies enabled us to finally “see” how LAG3 proteins are organized on the surface of T cells. Subsequent structural analyses revealed multiple potential targets for LAG3 antagonist drugs, including a potent neutralizing epitope linked to dual-inhibition of FGL1 and MHCII binding.

REFERENCE