Abstract

to the PhD thesis titled

Structural and functional characterisation of BTNL2: an orphan T cell coinhibitory molecule

by

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The activation of naïve T cells is a two-step process: the first, antigen-dependent step is mediated by engagement of the T cell receptor present on the T cell with the cognate peptide-major histocompatibility complex (pMHC) expressed on the antigen presenting cell (APC). However, this signal by itself is unable to drive the activation of naïve T cells. The second, antigen-independent step, is driven by the interaction of a T cell costimulatory receptor expressed on the T cell surface with its partner protein expressed on the APC. For example, post TCR-pMHC engagement, the binding of CD28 (on the T cell) to B7-1 or B7-2 (on the APC) leads to activation, proliferation and differentiation of the hitherto naïve T cell. Subsequently, to restore homeostasis, a T cell coinhibitory receptor binds to its cognate ligands expressed on the APC and results in T cell suppression. Binding of T cell coinhibitory receptors CTLA-4 and PD-1 to B7-1/B7-2 and PD-L1/PD-L2 respectively leads to such coinhibitory signaling. T cell costimulation and coinhibition is a complex orchestrated process involving the synchronized interaction of various receptor-ligand pairs. Although several such immunomodulatory receptors and their cognate ligands are well characterized, there still remain certain emerging proteins with T cell modulatory role who are less characterized. The structural and functional characterization of such immunomodulatory proteins can be used in the designing of novel therapeutic strategies to modulate immune response, both in context of cancer as well as autoimmune diseases.

BTNL2, a member of the butyrophilin-like family of proteins, is a T cell coinhibitor. However, neither its structure nor its binding partner(s) have been identified. The work described in this thesis covers the structural and functional characterization of the N-terminal ectodomain of mouse BTNL2. BTNL2 forms inclusion bodies when overexpressed in bacterial system, which is refolded *in vitro* and purified. Solution state biophysical characterization was done by size exclusion chromatography, analytical ultracentrifugation and one-dimensional proton NMR.

Flow cytometry-based cell recognition assays showed that refolded BTNL2 binds both B cells and PMA/ionomycin activated T cells from mouse splenocytes, which are known to express its unidentified binding partner. Further, it suppresses IL-2 production by *in vitro* activated $CD4^+$ T cells, thus demonstrating its T cell coinhibitory nature. These results, for the first time, demonstrate that the N-terminal IgV domain alone is capable of recapitulating the T cell inhibitory properties of BTNL2.

Further, the ensemble structure of BTNL2 has been solved using solution NMR spectroscopy. It adopts a beta-sandwich fold typical for IgV domains. NMR relaxation studies for the protein backbone show that it is a fairly rigid protein with a few flexible loops. Significantly, this is the first reported structure from the entire BTNL family across all species.

The current work describes structural and functional characterization of the N-terminal ectodomain of mouse BTNL2, and provides insight into identification of its binding partner.

Keywords: Immunoglobulin superfamily protein, immunoglobulin fold, immune receptors, T cell coinhibition, protein refolding, protein structure, NMR spectroscopy, flow cytometry