Immune Modulation by Butyrophilin 1A1 (BTN1A1)

Jinghua Wang, Brian Manick, Guoping Wu, Vassili Kalabokis | R&D Systems, Inc., 614 McKinley Place NE, Minneapolis, MN, 55413

Abstract

The butyrophilins are B7-like T cell co-regulatory molecules. These molecules are of increasing interest in cancer immunotherapy as they may represent a novel subset of immune checkpoint regulators. In this study, we expressed the extracellular domain of recombinant human butyrophilin 1A1 with a C-terminal 6-His tag (BTN1A1) in a NS0 mouse myeloma cell line, and investigated the biological functions of the purified recombinant BTN1A1 protein in vitro. Human T cells were treated with plate-bound anti-CD3 and BTN1A1 and the levels of multiple cytokines in the cell culture supernatants were measured using R&D Systems® Proteome Profiler™ Human Cytokine Array, and then subsequently confirmed by measuring individual cytokines with the appropriate Quantikine® ELISA Kits. BTN1A1 significantly decreased T cell-derived cytokines such as IL-2, IL-17E/IL-25, IL-21, CD40 Ligand, and C5a, but not IFN-γ. BTN1A1 also markedly inhibited anti-CD3-induced human T cell proliferation in a dose-dependent manner. BTN1A1 showed binding to anti-CD3 activated, but not resting T cells, and inhibited the differentiation of naive CD4+ T cell to regulatory T cells. Moreover, recombinant human BTN1A1 inhibited p38 MAPK, CREB, and TOR phosphorylation, suggesting that BTN1A1 modulates TCR signaling through the p38 MAPK, CREB, and TOR pathways. Taken together, our data suggests that BTN1A1 acts as a co-inhibitory molecule to modulate T cells through an unknown receptor on the surface of T cells. BTN1A1 may be a potential target of immune checkpoint molecules for therapeutic purposes.

Introduction

Butyrophilins (BTNs) belong to the immunoglobulin (Ig) superfamily of transmembrane proteins and share structural homology with B7 family members at the extracellular domain level. The human BTN family has been shown to have 13 members: BTN1A1, BTN2A1, BTN2A2, BTN2A3, BTN3A1, BTN3A2, BTN3A3, butyrophilin-like protein 2 (BTN2L2), BTN3L, BTN8L, BTN9L, BTN1L0 and SKINT-like (SKINTL). BTNs modulate T cell co-stimulatory responses, and are involved in T cell selection, differentiation, and cell fate determination. BTNs represent a new area of investigation for the design of future strategies aimed at modulating the immune system.1

BTN1A1, a 55-kDa type I transmembrane glycoprotein, is 494 amino acids (aa) long, and composed of an extracellular domain (ECD; aa 27–242), a transmembrane domain, and a cytoplasmic tail (aa 270–526) that contains the B30.2 domain. The BTN1A1 ECD displays two predicted IgV and IgC domains as do B7 and SKINT proteins, which interact with other Ig superfamily members.2 BTN1A1, the first butyrophilin identified, is required for the formation, secretion, and stabilization of milk fat globules.3 Growing evidence subsequently suggested that BTN1A1 may play various roles in the immune system. BTN1A1 has an inhibitory effect on T cell proliferation in vitro, and reduces expression of cytokines associated with T cell activation such as IL-2 and IFN-γ.4,5 Furthermore, BTN1A1 has a protective effect against the development of experimental autoimmune encephalomyelitis (EAE) in vivo.5 Lastly, staining with BTN1A1-Fc proteins has suggested the presence of unidentified receptors on T cells.6

BTNs are structurally related to B7 proteins, and are functionally implicated in immune regulation.7 Using recombinant BTN1A1, we demonstrate that BTN1A1 inhibits the differentiation of naive CD4+ T cells to regulatory T cells, and cytokine secretion by activated T cells, as well as p38 MAPK, CREB, and TOR pathways. Our data provide additional support of the notion that BTNs constitute a new family of T cell co-stimulatory/co-inhibitory molecules.

References


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