Multimeric Soluble 4-1BBL as a T Cell Stimulator for Adoptive Immunotherapy

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Abstract

Members of the TNF SuperFamily of ligands (TNFSFs) have significant potential as immuno-oncology agents. The TNFSFs are trimeric membrane proteins that can be cleaved into soluble single trimers. While the soluble single trimers can be easily prepared and studied, they have little or no activity in vivo. This deficiency is caused by the need to cluster their cognate receptors in the plane of the membrane in order to induce a supramolecular signaling complex on the cytoplasmic side of the plasma membrane. For the TNFSF ligands, this requires that they be used as many-trimer multimers that mimic the natural expression of many trimers on the surface of stimulating cells. To meet this need, we prepared fusion proteins comprised of the extracellular domains of TNFSF ligands joined to a natural protein that provides a multimerization scaffold. When surfactant protein D (SP-D) is used as a scaffold, the result is a 4-trimer TNFSF ligand product (UltraLigand™). Our published studies have described such multimeric forms of CD40L, OX40L, GITRL, CD27L/CD70, BAFF, RANKL, and TRAIL and shown that they are highly active in vitro and in vivo. As an extension of this work, 4-trimer forms of murine and human 4-1BBL (CD137L, TNFSF9) were constructed and expressed in CHO cells. As a co-stimulatory molecule, SP-D-4-1BBL (Ultra4-1BBL) activated both CD4+ and CD8+ T cells in vitro. Given the interest in 4-1BB (CD137) as a marker of therapeutically effective tumor-infiltrating lymphocytes (TILs), SP-D-4-1BBL should be a useful growth factor for TIL manufacturing and T cell culturing in general.

Receptor clustering is needed for 4-1BB (CD137) activation

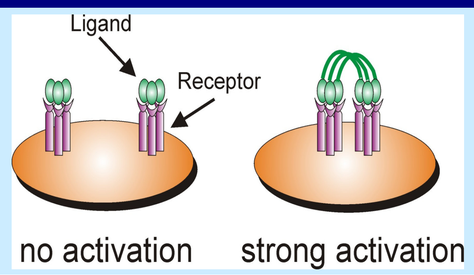

Fig. 1. Receptor clustering is needed for TNFRSF activation. 4-1BB (CD137), CD40, GITR, CD27, DR3, DR5, and Fas are some of the TNFSF receptors known to require clustering in order to trigger downstream signaling. Clustering can be achieved by an antibody mounted on FcRs on an adjacent cell or, as shown in this poster, by a many-trimer form of their TNFSF ligand.
Experimental evidence that the 4-1BB receptor must be cross-linked to signal comes from Rabu et al. These authors reported that a 1-trimer form of human 4-1BBL had no activating effects on human T cells whereas cross-linking the protein into 2- or more trimers led to a strongly activating protein. (Rabu, C., et al. Production of recombinant human trimeric CD137L (4-1BBL) - cross-linking is essential to its T cell co-stimulation activity. J Biol Chem. 280:41472-81, 2005).

Molecular design of 2- and 4-trimer TNFSF ligands

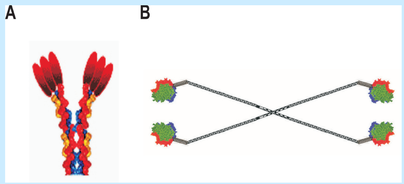

Fig. 2. Designing many-trimer forms of TNFSF ligands. The extracellular domain (ECD) of a TNFSF ligand can be genetically fused to one of two protein scaffolds, Acrp30 (Panel A) or surfactant protein D (SP-D) (Panel B). Following expression of the protein in CHO cells or 293 cells, 2-trimer or 4-trimer proteins are produced. The 2-trimer Acrp30-TNFSF ligand is called a Mega-Ligand™, whereas the 4-trimer SP-D-TNFSF ligand is called an Ultra-Ligand™. Commercially available proteins include MegaCD40L™, murine MegaOX40L™, MegaAPRIL™, MegaTNF™, and MegaFasL™. Multimeric is currently developing human MegaOX40L™ and MegaCD40L™ for advancement into clinical trials.

Murine SP-D-4-1BBL stimulates CD3-activated CD4+ T cells

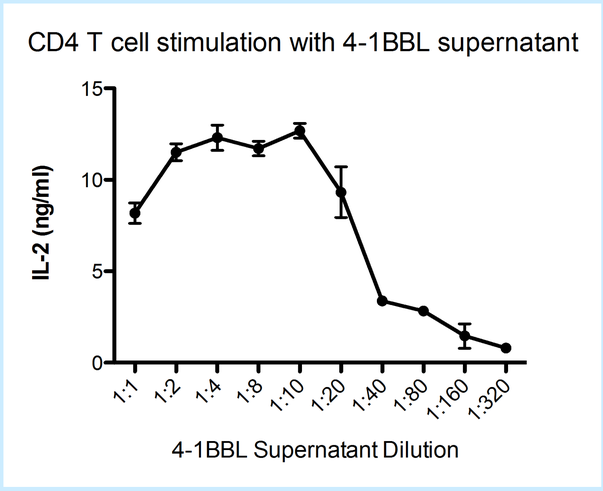

Fig. 3. Co-stimulation of murine CD4+ T cells by murine SP-D-CD40L (UltraCD40L). CD4+ T cells were isolated from mouse spleen by immunomagnetic beads and cultured with plate-bound anti-CD3 antibody along with dilutions of murine SP-D-4-1BBL (Ultra4-1BBL). IL-2 production was measured by ELISA. The initial SPD-4-1BBL CHO cell supernatant contained 2.8 ug/ml of soluble 4-1BBL by ELISA. The EC_{50} was about 125 ng/ml.

Data from Shravan Madireddi and Mick Croft,
La Jolla Institute of Immunology

Murine SP-D-4-1BBL stimulates CD3-activated CD8+ T cells

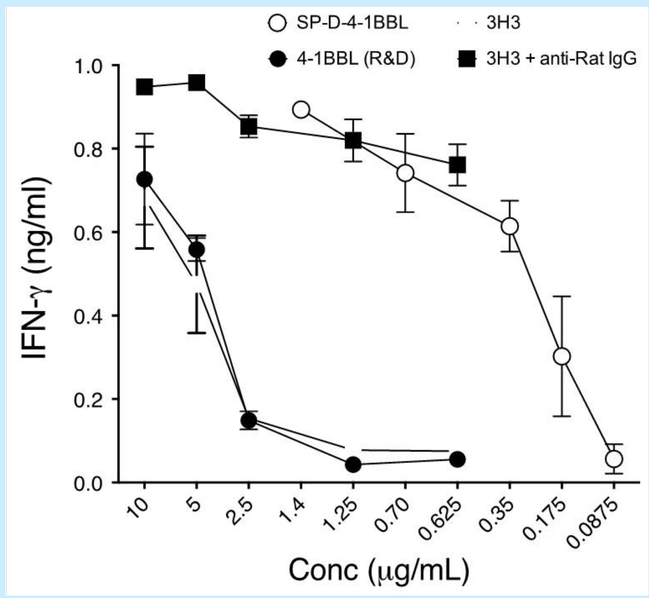

Fig.4. Co-stimulation of murine CD8+ T cells by 4-trimer murine SP-D-4-1BBL but not by 1-trimer 4-1BBL. CD8+ T cells were isolated from mouse spleen by immunomagnetic beads and cultured with plate-bound anti-CD3 antibody along with dilutions of murine SP-D-4-1BBL (Ultra4-1BBL). IFN-γ production was measured by ELISA. 3H3 rat anti-mouse 4-1BB antibody (□) was essentially inactive unless cross-linked by a secondary anti-rat IgG antibody (■). Likewise, a his-tagged 1-trimer form of 4-1BBL (R&D) (●) was essentially inactive. However, 4-trimer murine SP-D-4-1BBL was highly active as a single soluble molecule (○). The EC_{50} for SP-D-4-1BBL was about 300 ng/ml.

Data from Shravan Madireddi and Mick Croft,
La Jolla Institute of Immunology

Human SP-D-4-1BBL co-stimulates human T cells in vitro

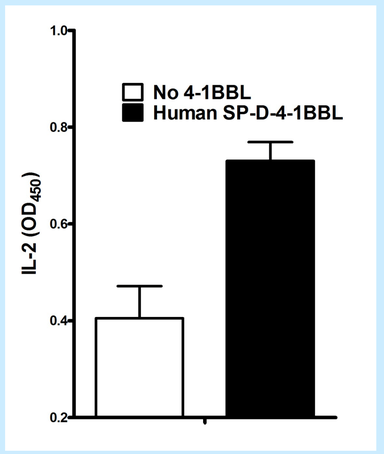

Fig. 5. Human SP-D-4-1BBL co-stimulates human T cells in vitro. PBMCs were cultured on plastic plates coated with anti-CD3 antibody without or with human SP-D-4-1BBL in the media. After 3 days, supernatants were assayed for IL-2 production by ELISA. As shown, SP-D-4-1BBL is a strong activator of TCR-stimulated T cells in culture.

Uses for many-trimer SP-D-4-1BBL

Adoptive Immunotherapy:

SP-D-4-1BBL (Ultra4-1BBL) will be useful for growing large numbers of Tumor-Infiltrating Lymphocytes (TILs) ex vivo for subsequent adoptive immunotherapy.

NK cells can also be grown in culture using 4-1BBL.

***In Vivo* Immunotherapy:**

SP-D-4-1BBL (Ultra4-4BBL) will be interesting to test as a costimulatory molecule for cancer immunotherapy and vaccines. It will likely have a different toxicity profile than the anti-4-1BB antibody that had significant toxicity in clinical trials.

Acknowledgements

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For publications, see www.multimericbio.com

Patent protection for multimeric TNFSFs (MegaLigands™ and UltraLigands ™) is provided by US 7,300,774B1, US 7,332,298B2, EP01246925B1, US 2009/0081157A1, and related counterparts worldwide.