Mouse CD229 Ligation Co-stimulates T Cell Activation

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ABSTRACT

CD229/SLAMF3 is a transmembrane glycoprotein primarily found on mature T cells, B cells, myeloid cells, macrophages, and thymocytes. The functions of CD229 remain uncertain. Data from CD229 knockout mice indicate that CD229 is uniquely involved in enhancing T cell activation. However, co-ligation of CD229 and T cell receptor (TCR) using anti-CD229 (mAb) and anti-CD3 antibodies has been demonstrated to inhibit T cell activation. We expressed the mouse CD229 extracellular domain (aa 48-454) with a poly-Histidine tag in the NS0 mouse myeloma cell line and investigated the functions of purified recombinant CD229 in mouse CD3$^+$ cells *in vitro*. Concurrent ligation of CD229 and TCR with immobilized CD229-His protein and anti-CD3 antibody significantly enhanced cell proliferation and IFN-γ secretion in mouse CD3$^+$ splenocytes in a dose-dependent manner. Furthermore, pre-treatment of CD3$^+$ splenocytes with polyclonal anti-CD229 completely abolished the co-stimulative actions of CD229-His. This suggests that CD229-His co-stimulates T cells through CD229 receptors on T cell surfaces. Moreover, ligation of CD229 with CD229-His in CD3$^+$ cells led to ERK1/2 phosphorylation in response to anti-CD3 stimulation. Taken together, these results suggest that CD229 acts as a homophilic co-stimulatory molecule and is able to up-regulate T cell activation.

INTRODUCTION

The signaling lymphocyte activation molecule (SLAM) family of immune cell receptors is closely related to the CD2 family of the immunoglobulin (Ig) superfamily of molecules. The SLAM family includes nine members, known as SLAM/SLAMF1, CD48/SLAMF2, CD229/SLAMF3, 2B4/SLAMF4, CD84/SLAMF5, NTB-A/SLAMF6, CRACC/SLAMF7, BLAME/SLAMF8, and CD2F-10/SLAMF9.[1] CD229, also known as SLAMF3 and Ly9, is a 120 kDa type I transmembrane glycoprotein. Mature mouse CD229 consists of a 406 amino acid (aa) extracellular domain (ECD) containing two IgG V-like and two IgG C-like regions, a 21 aa transmembrane segment, and a 180 aa cytoplasmic domain with two immunoreceptor tyrosine-based switch motifs (ITSMs).[2] CD229 is expressed on T cells, B cells, NK cells, myeloid cells, thymocytes, and monocytes.[3] Homophilic binding between CD229 molecules is mediated through its IgG V-like domains.[4] SLAM-associated protein (SAP) is shown to be necessary for CD229 tyrosine phosphorylation in T lymphocytes. Upon activation, phosphorylated CD229 interacts with the SH2 domain of GRB2 in T cells. The CD229–GRB2 interaction regulates receptor internalization in a PI 3-Kinase-dependent manner and controls CD229 levels on the cell surface.[5]

In spite of accumulating data on CD229, the exact functions of this receptor remain uncertain. Studies have shown that ligation of CD229 with a monoclonal antibody (mAb) against mouse CD229 inhibits T lymphocyte activation. Cross-linking of CD229 with an anti-CD229 mAb down-regulates the expression of CD69 and CD25 on anti-CD3 activated T cells.[2] Furthermore, CD229 ligation with anti-CD229 mAb reduces the secretion of cytokines, including IFN-γ, IL-2, IL-4, IL-6, IL-10, and TNF, by anti-CD3 activated T cells.[3] These observations suggest that CD229 is a co-inhibitory molecule. However, studies using CD229 knockout mice show that in the absence of CD229, the levels of IL-2 and IL-4 are reduced after T cell activation, indicating that CD229 functions as a positive regulator of T cell activation.[6] In this study, using *in vitro* experiments with a newly generated soluble mouse CD229 ectodomain, we examined the functional role of CD229 in T cells.

RESULTS

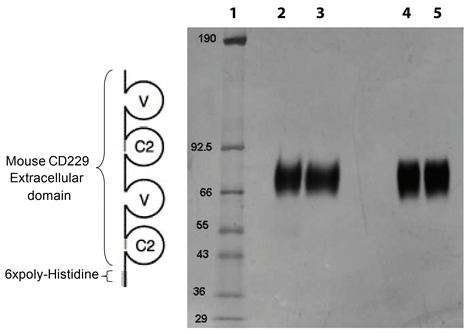

Figure 1. Diagram of Recombinant Mouse CD229-His (rmCD229-His, aa 48-454), and SDS-PAGE analysis of two lots of rmCD229-His under reducing and non-reducing conditions. The protein bands were visualized by standard silver staining. Lane 1: Molecular weight marker; Lanes 2 & 3: Two lots of rmCD229-His under reducing conditions; Lanes 4 & 5: Two lots of rmCD229-His under non-reducing conditions.

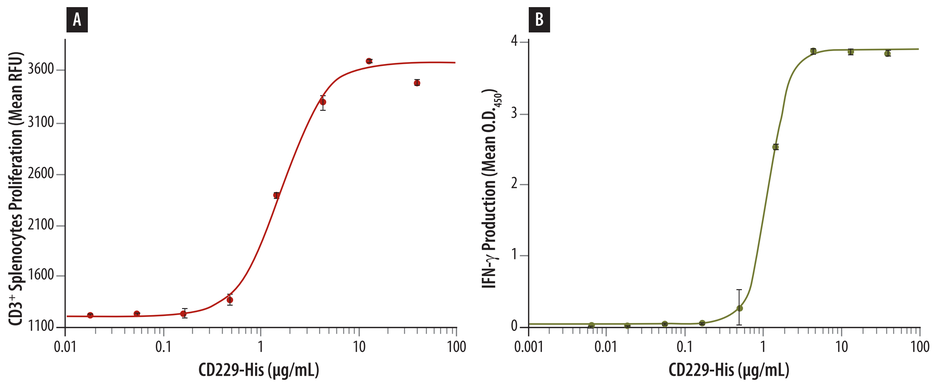

Figure 2. CD229 enhances anti-CD3-induced cell proliferation and IFN-γ secretion in mouse CD3$^+$ splenocytes in a dose-dependent manner. Splenocytes were harvested from BALB/c mice. Mouse CD3$^+$ T cells were isolated using the MagCellect™ Mouse CD3$^+$ T Cell Isolation Kit (Catalog # MAGM201) with routine purity >95%. Purified CD3$^+$ T cells were incubated with an immobilized Hamster Anti-Mouse CD3ε Monoclonal Antibody (100 ng/mL, Catalog # MAB484) and increasing concentrations of Recombinant Mouse CD229-His (Catalog # 2555-CD) for 72 or 48 hours. **A.** Cell proliferation was assessed by a fluorometric assay using the redox-sensitive dye Resazurin (Catalog # AR002). **B.** IFN-γ secretion was measured using the Mouse IFN-γ Quantikine® ELISA Kit (Catalog # MIF00).

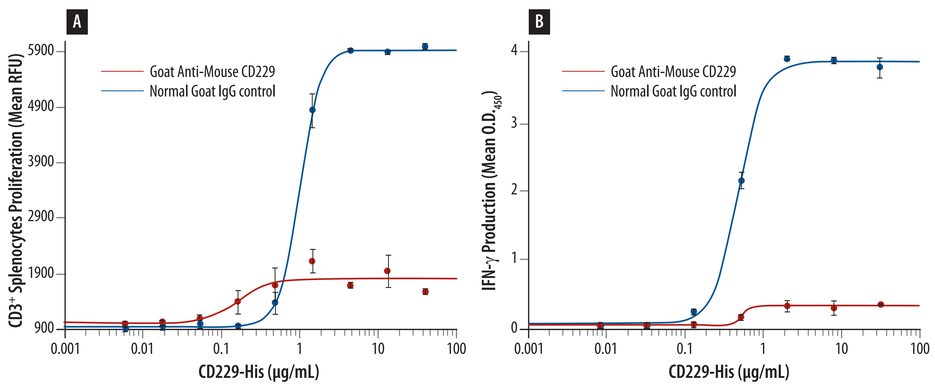

Figure 3. Pretreatment of mouse CD3$^+$ cells with goat anti-mouse CD229 abolishes the stimulatory effects of rmCD229-His on T cells. To block CD229-dependent signaling, cells were pretreated with 10 µg/mL Goat Anti-Mouse CD229 Affinity-purified Polyclonal Antibody (Catalog # AF2555) or Normal Goat IgG Control (Catalog # AB-108-C) for 1 hour before *in vitro* cell stimulation with Recombinant Mouse CD229-His (Catalog # 2555-CD) and anti-CD3 antibody. **A.** Cell proliferation was assessed by a fluorometric assay using the redox-sensitive dye Resazurin (Catalog # AR002). **B.** IFN-γ secretion was measured using the Mouse IFN-γ Quantikine ELISA Kit (Catalog # MIF00).

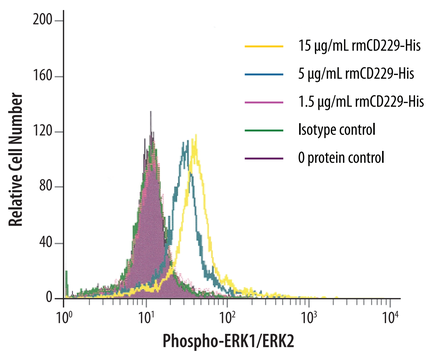

Figure 4. CD229 ligation results in a dose-dependent increase of phosphorylated ERK1/2 in the presence of an anti-CD3 antibody. Mouse CD3$^+$ T cells were stimulated with an immobilized Hamster Anti-Mouse CD3ε Monoclonal Antibody (100 ng/mL, Catalog # MAB484) and the indicated concentrations of Recombinant Mouse CD229-His (Catalog # 2555-CD) at 37 °C, 5% CO_2 for 1 hour. Cells were then washed, immediately fixed, and washed again. The cells were permeabilized using the Flow Cytometry Fixation & Permeabilization Buffer Kit (Catalog # FC009). After washing, the cells were blocked with a Rat Anti-Mouse Fcγ RII/III (CD32/CD16) Monoclonal Antibody (Catalog # MAB1460) for 20 minutes before the addition of a Fluorescein-conjugated Rabbit Anti-Phospho-ERK1/ERK2 Affinity-purified Polyclonal Antibody (Catalog # IC1018F) or a Fluorescein-conjugated Rabbit IgG Control (Catalog # IC105F) for an additional 45 minutes, at room temperature in the dark. ERK1/2 activation was detected by flow cytometry.

SUMMARY

- Treatment of mouse CD3$^+$ splenocytes with recombinant mouse CD229 (rmCD229) induces cell proliferation and IFN-γ secretion in a dose-dependent manner.
- Pre-treatment of CD3$^+$ splenocytes with a polyclonal anti-CD229 antibody abolishes rmCD229-induced cell proliferation and IFN-γ production.
- Stimulation of CD3$^+$ splenocytes with anti-CD3 and rmCD229 results in ERK1/2 phosphorylation.
- These data strongly suggest that CD229 acts as a homophilic molecule capable of up-regulating T cell activation.

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