Appropriate cross-linking is required for co-stimulatory activity of human anti-CD27 antibody in a transgenic mouse model

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Abstract

CD27 is a member of TNFR superfamily. It is constitutively expressed on the majority of T cells and a subset of NK cells, playing key roles in T cell activation and survival and in NK cell proliferation and cytotoxicity upon interaction with ligand CD70. Some antibodies to mouse CD27 have been reported that display agonistic and anti-tumor activities while other mAbs had less anti-tumor activity and were depleting. We hypothesized that differences in these antibodies may be due to Fc receptor engagement, as has recently been shown for the adjuvant and anti-tumor activities of agonistic mouse CD40 mAbs, which is also member of TNFR superfamily. We have developed and previously described a human anti-human CD27 antibody (1F5) and a human CD27 transgenic mouse model (hCD27-Tg) to explore the therapeutic potential of targeting CD27. In this study, we examined the effect of modifying the constant regions of the 1F5 mAb on its ability to enhance antigen specific T cell responses. With the original 1F5 hG1 as template, a panel of 1F5 variants was made including 1F5 mG1, 1F5 mG2a, 1F5 mG1$_{D265A}$ and 1F5 hG1$_{N297S}$ using molecular cloning techniques. All of the variants retained equal binding to hCD27 as shown by ELISA and flow cytometry studies. In addition, Biacore analysis confirmed the expected pattern of binding to human and mouse FcγRs. Co-injection of 1F5 or its variants with ovalbumin enhanced antigen-specific CD8 T cell response to different extents, as detected by SIINFEKL-specific IFNγ-ELISPOT and ICS. The 1F5 mG1 induced the highest number of IFNγ-producing CD8$^+$ cells, whereas 1F5 mG2a was relatively weaker at enhancing the CD8 T cell response. The hIgG1 version of 1F5 was intermediate in activity. Introduction of the D265A mutation that disrupts FcγR binding into the mIgG1 eliminated the co-stimulatory function of 1F5. Similarly, the 1F5 hG1$_{N297S}$ was not significantly better than irrelevant control human IgG1(clgG1). The isotype-specific effects on our anti-hCD27 mAb are surprisingly consistent with the findings described for the agonist anti-mCD40 mAbs (*A.L. White et al. J Immunol 187:1754, 2011; F. Li and J.V. Ravetch. Science 333:1030, 2011)*, and imply that engagement of the inhibitory Fcγ receptors (FcγRIIb) is driving the co-stimulatory activity in this model. Interestingly, the 1F5 hG1 triggered a significant T cell response, despite the lack of FcγRIIb binding by Biacore analysis. The effect of these variants on anti-tumor activity in hCD27 transgenic mice is currently being investigated. The 1F5 hIgG1 mAb (CDX-1127) is currently undergoing clinical evaluation in a phase 1 trial of patients with advanced cancers.

CD27 Background

- Member of the tumor necrosis factor (TNF) receptor superfamily
- Constitutively expressed on the majority of mature T cells, memory B cells, and a portion of natural killer (NK) cells
- CD27/CD70 Co-stimulatory Pathway
  - CD27 activation well-regulated by CD70; ligand is generally only transiently expressed on activated T cells, B cells, and dendritic cells
  - On T cells: causes activation, proliferation, survival, and maturation of effector capacity and memory
  - On human B cells: activates and promotes the generation of plasma cells, proliferation, and the production of immunoglobulin
  - On NK cells: induces cytolytic activity
- In vivo CD27 stimulation with its ligand (CD70) promotes strong primary and secondary CD8+ cytotoxic T cell responses and expression of CD70 on dendritic cells improves immunity of dendritic cell vaccines (*Rowley TF and Al-Shamkhani A, J Immunol. 2004; Keller AM et. al. Immunity 2008*)
- Agonist anti-CD27 mAbs can induce potent anti-tumor immunity through T cell activation *(French, RR et al. Blood 2007; Sakanishi, T. et al. BBRC 2010; Roberts, DJ et al. J. Immunotherapy 2010)*

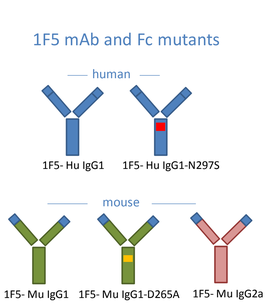
Reagents and model

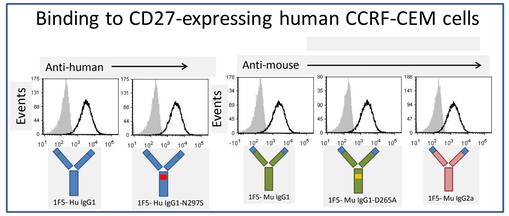
1F5 human anti-huCD27 mAb

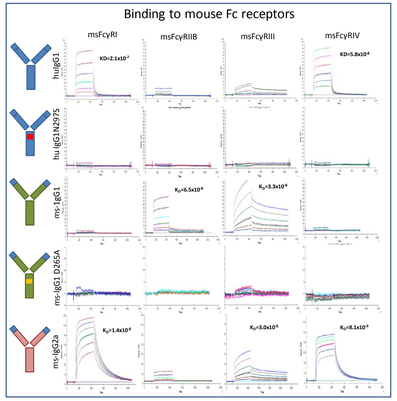
- Generated from human Ig expressing mice
- High specificity and affinity for human and macaque CD27
- Agonist mAb; induces T cell activation and proliferations in vitro when combined with TCR stimulation

C57Bl/6 huCD27 Tg mice

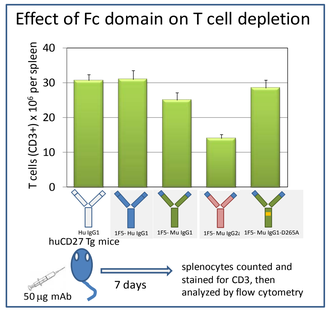
- BAC clone containing the CD27 gene was used for microinjection of mice embryos
- Transgenic lines were established and characterized for appropriate expression and regulation of hu CD27
- huCD27 Tg mice were fully backcrossed to C57Bl/6 strain

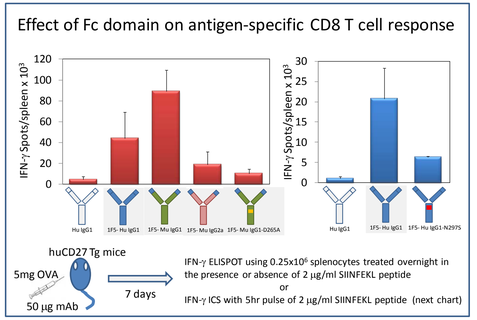


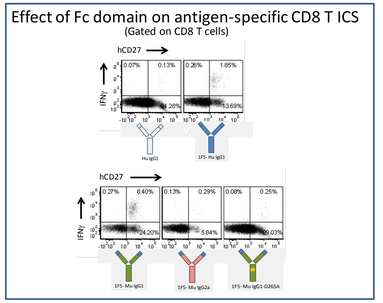


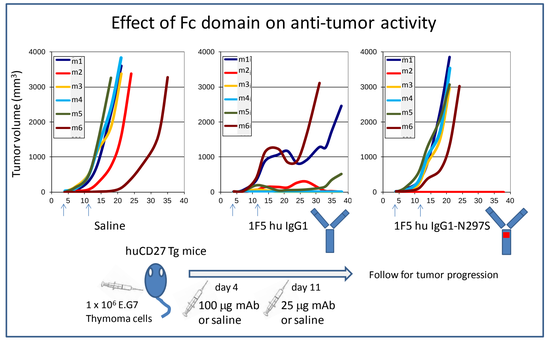


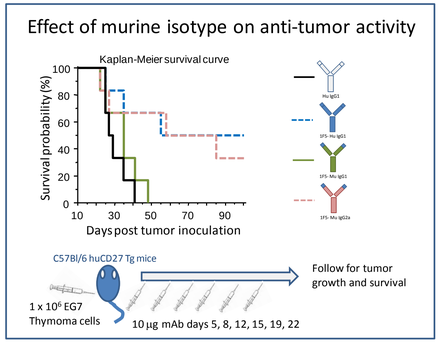
Results











Summary

- We constructed Fc variants of the human anti-huCD27 mAb 1F5 that retained CD27 binding and resulted in expected FcR binding patterns
- FcR interaction is essential for co-stimulatory activity of 1F5 mAb
- The ms IgG1 isotype induced significantly greater antigen specific T cell response than ms IgG2a
- The ms IgG2a induced significantly greater anti-tumor response than ms IgG1
- Additional studies are being performed to better understand the FcR interactions required for the functional activities of 1F5 mAb

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