

Anti-CD137 mAb Deletes Both Donor CD4⁺ and CD8⁺ T Cells in Acute Graft-versus-host Disease

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We previously demonstrated that *in vivo* engagement of CD137, a member of TNF receptor superfamily, can delete alloreactive CD4⁺ T cells through the induction of activation-induced cell death (AICD) in chronic graft-versus-host disease (cGVHD) and subsequently reverse established cGVHD. In this study, we further showed that agonistic anti-CD137 mAb was highly effective in triggering AICD of donor CD8⁺ T cells as well as donor CD4⁺ T cells in the C57BL/6→unirradiated (C57BL/6 × DBA/2)F1 acute GVHD model. Our results suggest that strong allostimulation should facilitate AICD of both alloreactive CD4⁺ and CD8⁺ T cells induced by CD137 stimulation. Therefore, depletion of pathogenic T cells using agonistic anti-CD137 mAb combined with potent TCR stimulation may be used to block autoimmune or inflammatory diseases mediated by T cells.

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CD137 is a member of the TNF receptor family and functions as a costimulatory molecule for T cells (1). Engagement of CD137 using anti-CD137 mAbs has been shown to effectively eradicate established tumors mainly through activation of CD8⁺ T cells (1). Paradoxically, however, anti-CD137 mAbs have strong immunosuppressive effects on a variety of autoimmune or inflammatory diseases that are believed to be mediated mainly by CD4⁺ T cells (2). A consensus on how stimulation of CD137 prevents disease has yet to emerge, but CD137 appears to be involved in the hyperactivation of T cells, causing them to acquire regulatory capacity or induce

cell death (3).

In the DBA/2→unirradiated (C57BL/6 × DBA/2)F1 (BDF1) chronic graft-versus-host disease (cGVHD) model, anti-CD137 mAb is highly effective in inhibiting cGVHD by deleting donor CD4⁺ T cells which are required for breaking host B-cell tolerance (4). In a more clinically relevant cGVHD model, anti-CD137 mAb reverses skin fibrosis, ulceration, and alopecia, a dominant feature of cGVHD, ultimately improving a general health condition (5). The reversal is associated with increased apoptosis of donor CD4⁺ T cells. The Fas death pathway is required for activation-induced cell death (AICD) of alloreactive CD4⁺ T cells.

In this study, we hypothesized that anti-CD137 mAb might induce AICD of alloreactive CD8⁺ T cells as well as CD4⁺ T cells if they received strong allostimulation. We chose the C57BL/6→unirradiated BDF1 acute GVHD (aGVHD) model as a model system to test this hypothesis, since strong alloimmunity for donor CD4⁺ and CD8⁺ T cells occurs in this disease model. BDF1 mice received C57BL/6 T cells and anti-CD137 mAb (3H3) immediately after the cell transfer. FACS analysis showed that there was a marked increase in apoptosis of both splenic CD4⁺ and CD8⁺ T cells in anti-CD137-treated mice 5 days after the cell transfer (Fig. 1A). A majority of donor CD4⁺ and CD8⁺ T cells expressed low levels of CD62L in both control Ig- and anti-CD137-treated mice (Fig. 1B), indicating that AICD caused their apoptosis following injection of anti-CD137 mAb, as seen previously (4). At this time point, a higher percent of donor CD4⁺ T cells underwent activation and apoptosis following administration of an-

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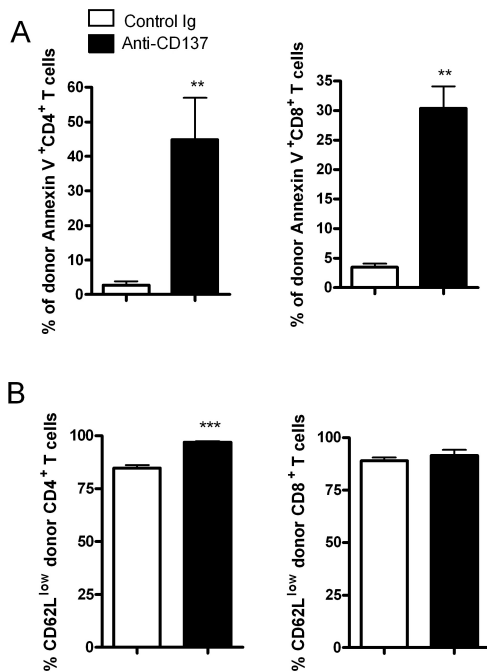


Figure 1. Anti-CD137 mAb induces apoptosis of both donor CD4⁺ and CD8⁺ T cells in aGVHD. aGVHD was induced by transferring 5 × 10⁷ C57BL/6 spleen/lymph node cells into BDF1 mice. Immediately thereafter, anti-CD137 mAb or control Ig (200 μg per mouse) was injected. Splenocytes were analyzed by flow cytometry 5 days after parental cell transfer. (A) Percent of Annexin V⁺ donor CD4⁺ and CD8⁺ T cells. (B) Percent of CD62L^{low} donor CD4⁺ and CD8⁺ T cells. n=3 mice per group. **p<0.01 and ***p<0.001 between the 2 groups.

ti-CD137 mAb, as compared with donor CD8⁺ T cells (Fig. 1). This result may indicate that donor CD4⁺ T cells had a more rapid kinetics for their activation and subsequent AICD in response to anti-CD137 mAb.

A long-term observation demonstrated that control Ig-treated mice experienced severe loss of body weight and their mortality rate was high (70%), whereas anti-CD137-treated mice maintained normal body weight and stayed healthy until the termination of experiments (Fig. 2). FACS analysis for splenocytes showed that administration of anti-CD137 mAb prevented severe lymphodepletion in the spleen, a parameter for aGVHD (Table I). Anti-CD137-mediated inhibition of aGVHD was due to the deletion of donor CD4⁺ and CD8⁺ T cells and a subsequent failure of donor cell engraftment (Table I).

In two preclinical models of cGVHD, now it is clear that agonistic anti-CD137 mAb has the ability to delete pathogenic alloreactive CD4⁺ T cells and autoreactive B cells. However, there is evidence showing that agonistic anti-CD137 mAb can delete antigen-specific CD8⁺ T cells as well as CD4⁺ T cells *in vivo* (6,7). Earlier treatment with agonistic anti-CD137 mAb maintains elevated levels of TNF-α in lymphocytic choriomeningitis virus (LCMV)-infected mice, leading to Fas expression on activated CD8⁺ T cells and this in turn results in Fas-mediated apoptosis (6). Even though Fas-mediated death signal is not sufficient to delete LCMV antigen-specific CD8⁺ T cells, STAT3 activation by signaling through CD137 in dendritic

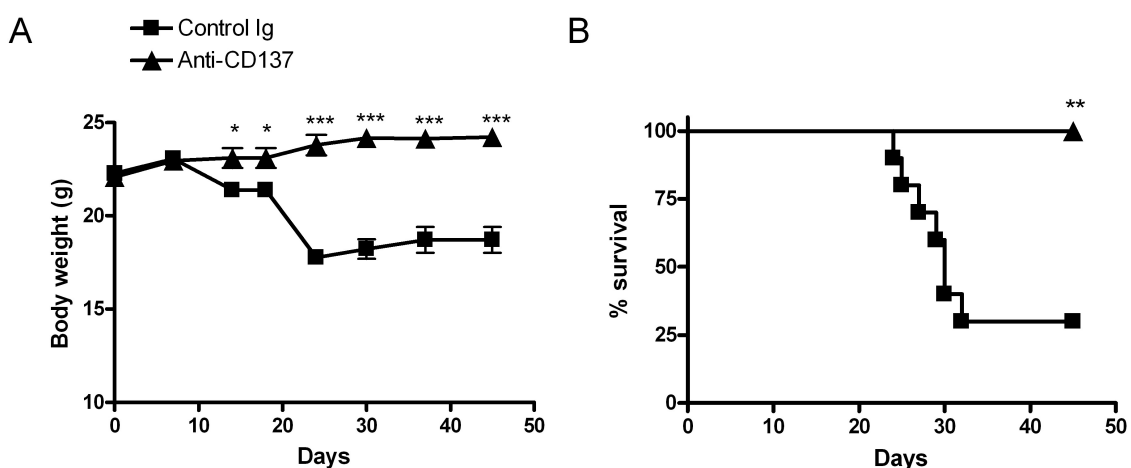


Figure 2. Anti-CD137 mAb completely blocks aGVHD. aGVHD was induced by transferring 5 × 10⁷ C57BL/6 spleen/lymph node cells into BDF1 mice. Immediately thereafter, anti-CD137 mAb or control Ig (200 μg per mouse) was injected (n=10 per group). (A) Changes of body weight. *p<0.05 and ***p<0.001 between the 2 groups at the indicated time points. (B) Survival curve. **p<0.05 between the 2 groups.

Table I. Inhibition of donor cell engraftment by anti-CD137 mAb^a

Group	Total splenocytes	Number (percentage) of host cells		
		B cells	CD4 ⁺ T cells	CD8 ⁺ T cells
Control Ig	24.91 ± 0.43	0.40 ± 0.43 (1.73 ± 0.7)	2.29 ± 1.79 (4.38 ± 1.69)	0.75 ± 0.78 (3.35 ± 1.65)
Anti-CD137	110 ± 31.76	63.15 ± 18.35 (55.64 ± 1.94)	18.49 ± 4.74 (16.53 ± 1.48)	15.24 ± 4.51 (13.245 ± 1.27)
Number (percentage) of donor cells				
	Total cells	B cells	CD4 ⁺ T cells	CD8 ⁺ T cells
Control Ig	22.19 ± 23.14 (88.31 ± 4.61)	17.61 ± 18.81 (76.24 ± 7.85)	10.46 ± 10.37 (48.56 ± 3.73)	17.8 ± 18.31 (80.96 ± 1.35)
Anti-CD137	4.24 ± 22.77 (4.40 ± 1.89)	0.02 ± 0.01 (0.48 ± 0.19)	0 (0.17 ± 0.10)	0 (0.72 ± 0.64)

^aaGVHD was induced by transferring 5×10^7 C57BL/6 spleen/lymph node cells into BDF1 mice. Immediately thereafter, mice received control Ig or anti-CD137 mAb (200 μ g per mouse). Splenocytes were analyzed by flow cytometry 44 days after disease induction. Absolute number or percent of donor cells were counted by staining splenocytes with anti-H-2K^b plus anti-B220, anti-CD4 or anti-CD8 mAbs. ^bValues for total splenocytes and lymphocyte subsets are shown as (mean \pm SD) $\times 10^6$ (n = 10 mice per group).

cells is required for their complete AICD (7). In this study, we showed that agonistic anti-CD137 mAb could completely delete not only donor CD4⁺ T cells but also CD8⁺ T cells in the C57BL/6 \rightarrow BDF1 aGVHD model. As seen in cGVHD (4,5) it is likely that engagement of CD137 provides strong costimulatory signaling leading to AICD for donor CD4⁺ and CD8⁺ T cells that receive sustained allostimulation during the evolution of aGVHD. It remains to be elucidated whether other host hematopoietic and/or nonhematopoietic cells are needed for AICD of donor T cells by agonistic anti-CD137 mAb in GVHD.

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CONFLICTS OF INTEREST

The authors have no financial conflict of interest.

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