Review

Immunotherapy with Agonistic Anti-CD137: Two Sides of a Coin

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CD137 (4-1BB), a member of the TNF receptor superfamily, is an inducible T cell costimulatory receptor primarily expressed on activated $CD4^+$ and $CD8^+$ T cells. Agonistic monoclonal antibodies (mAbs) against CD137 greatly enhance T cell-mediated immune responses against many types of tumors and viruses. Surprisingly, these agonists also showed therapeutic effects in several autoimmune diseases. These findings suggest that in different disease environments, CD137 engagement with agonist mAb *in vivo* can diametrically modulate immune response outcomes. Therefore, CD137 agonists represent a promising immunotherapeutic approach to a wide array of disparate immune disorders. However, CD137's potency in modulating immune response necessitates caution when targeting CD137 clinically. *Cellular & Molecular Immunology*. 2004;1(1):31-36.

Key Words: CD137, agonistic antibody, immunotherapy, immune disorders

CD137 (4-1BB) is a member of the tumor necrosis factor receptor superfamily (TNFRSF9) and a T cell costimulatory receptor induced by TCR activation. In addition to its expression on activated T cells and NK cells (1-3), CD137 is also expressed on NKT cells (4), $CD4^+CD25^+$ regulatory T cells (5, 6) and myeloid cells, including monocytes, neutrophils and dendritic cells (7-10). Its natural ligand CD137L (4-1BBL) - a member of TNF superfamily (TNFSF9) - has been detected on professional APCs including B cells, macrophages, and dendritic cells (11-13). This expression pattern implies the possibility that CD137/CD137L interaction may be involved in multiple steps in various innate and adaptive immune responses.

It has been shown that signaling through CD137 by either CD137L or agonistic monoclonal antibodies (mAbs) against CD137 leads to increased TCR-induced T cell proliferation, cytokine production and functional maturation, and prolonged $CD8^+$ T cell survival (1, 14, 15). These effects result from: (1) the activation of the NF-κB, c-Jun NH_2-terminal kinase/stress-activated protein kinase (JNK/SAPK), and p38 mitogen-activated protein kinase (MAPK) signaling pathways (16-18) and (2) the control of anti-apoptotic- and cell cycle-related gene expression (15,19-21). Experiments performed in both CD137 and CD137L-deficient mice have additionally demonstrated the importance of CD137 costimulation in the generation of a fully competent T cell response (22-25). IL-2 and IL-15 activated NK cells express CD137, and ligation of CD137 by agonistic mAbs stimulates NK cell proliferation and IFN-γ secretion, but not their cytolytic activity. Furthermore, CD137-stimulated NK cells promote the expansion of activated T cells *in vitro* (26). In accordance with their costimulatory function, agonist mAbs against CD137 have been shown to promote rejection of cardiac and skin allografts, eradicate established tumors, broaden primary antiviral $CD8^+$ T cell responses, and increase T cell cytolytic potential (27-29). These studies support the view that CD137 signaling promotes T cell function which may enhance immunity against tumors and infection.

However, recent findings indicate a diametric role of CD137 signaling. CD137-specific agonist mAbs that enhanced tumor rejection in mice were also capable of suppressing T-dependent humoral immunity (30) and ameliorating both the antigen-induced organ-specific autoimmune disease experimental autoimmune encephalomyelitis (EAE) (31) and the spontaneous systemic autoimmune disease systemic lupus erythematosus (SLE) (32-34). Supporting the possible regulatory role of CD137 signaling in T cell function, it was shown that CD137-deficient T cells are more responsive to mitogens compared with WT T cells (35). These studies strongly suggest that 4-1BB signaling differentially modulates immune responses *in vivo*. Here, we review recent studies on the application of agonistic mAbs against CD137 in different disease models.

Tumors

Due to the role of CD137 signaling in promoting T cell and NK cell proliferation, IFN-γ secretion, and prolonging the survival of $CD8^+$ T cells, CD137 engagement may provide an attractive strategy for immunotherapy of cancer. mAbs against CD137 have variable anti-tumor therapeutic effects depending on the immunogenicity of the experimental tumor and anatomical site of tumor growth. Treatment with agonist anti-CD137 mAbs caused regression of large, well-established tumors in mice, including Ag104A sarcoma, P815 mastocytoma, EL4E7 lymphomas and B10.2 fibrosarcoma (28, 36, 37). This treatment also generated systemic antitumor effects in established intracranial tumors

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Received for publication Dec 30, 2003. Accepted for publication Jan 20, 2004.

including MCA sarcoma and GL261 glioma, but not in established subcutaneous and pulmonary tumors (38). Agonistic anti-CD137 treatment shows no antitumor effects against the established or metastasized poorly immunogenic tumors, including B16/D5 melanoma, C3 tumor, TC-1 lung carcinoma and B16-F10 melanoma (36, 38). The failure of anti-4-1BB mAbs in treating these tumors is due to immunological ignorance of tumor antigen-specific CTLs during the progressive growth of the tumors (36). Therefore, it seems that CD137 signaling is not beneficial for T cell priming or initiating an immune response, but rather acts on cells that have already been activated by tumor Ags *in vivo*. In support of this, Miller et al. found that initiation of anti-CD137 treatment relatively soon after tumor challenge was not as effective as initiation of treatment after the tumor had become more established (37). Breaking CTL ignorance by immunization with a tumor antigen-derived peptide is necessary for anti-CD137 mAbs to induce a sufficient CTL response to cause the regression of established tumors (36). The poor immunogenicity of human tumors may require that tumor antigen-derived peptides be given in concert with anti-CD137 treatment when using these mAbs to treat human cancers.

In addition to increasing the intensity of anti-tumor responses, CD137 treatment prolongs the survival of preactivated tumor-specific $CD8^+$ T cells both *in vitro and in vivo*, enhancing the efficacy of adoptive immunotherapy (38-40). Furthermore, anti-CD137 treatment can synergize with IL-12 gene therapy by greatly amplifying both CTL- and NK cell-mediated immune responses, leading to the complete regression of established MCA26 colon cancer in a hepatic metastasis model (41). Anti-CD137 treatment also synergizes with Flt3L treatment to promote the regression of B10.2 fibrosarcomas (37).

Another attractive method of CD137-targeted cancer immunotherapy is to modify tumor cells with membrane-bound single-chain Fv fragments (scFv) of anti-CD137 (42). Transfecting the weakly immunogenic mouse melanoma K1735 with scFv of anti-CD137 greatly increases its immunogenicity and induces protection and therapeutic effects against unmodified wild type tumors with similar or even better efficacy than agonistic anti-CD137 treatment.

The therapeutic effect of anti-CD137 is dependent on $CD8^+$ T cells, inducing increases in tumor-specific CTL activity and associated IFN-γ production. However, the requirement of $CD4^+$ T cells and NK cells varies depending on tumor model used (26, 28, 36-38). In some tumor models, such as the P815 mastocytoma, NK cells expressing CD137 are critical to trigger the therapeutic effects because of their immunoregulatory activity on $CD8^+$ CTL through producing IFN-γ (3, 26). IFN-γ is required for the therapeutic effect because it mediates the homing of effector T cells to the tumor site (43). scFv of anti-CD137 modified K1735 cells induce strong $CD4^+$ type 1 T-helper cell (Th1)-mediated antitumor responses involving both NK cells and macrophages. Although $CD4^+$ T cells are not required during the generation of the initial immune response, they might play a role in memory responses against some tumor models (37). The therapeutic effects of anti-CD137 mAbs are also dependent on their ability to prevent programmed cell death in $CD8^+$ cytolytic T lymphocytes (CTL), thus extending their operative life (40), and preventing and reversing established anergy of CTL *in vivo* (44). Therefore, CD137 costimulation may enhance anti-tumor immunity in multiple ways.

Viral infections

CD137 seems to play a role in $CD8^+$ T cell-mediated antiviral responses. CD137L-deficient mice had decreased CTL responses to influenza virus in the late stage of primary response and defective secondary response. They also exhibited diminished $CD8^+$ T cell responses and IFN-γ expression after lymphocytic choriomeningitis virus (LCMV) infection, and impaired the efficacy of vaccination with LCMV peptide in long term protection generation against LCMV infection (23-25, 45). The CD137-deficient mice showed decreased CTL activity against vesicular stomatitis virus (VSV). However, these mice showed normal humoral immune responses to viruses (35). These studies imply that CD137/CD137L interaction may play an important role in maintaining $CD8^+$ T cell responses and generating their memory responses. In support of this, Halstead et al. found that *in vivo* CD137 stimulation with an agonistic mAb enhanced and broadened the $CD8^+$ T cell response to influenza virus. It preferentially expanded $CD8^+$ T cells that recognized nondominant as compared with dominant epitopes and greatly enhanced direct *ex vivo* cytotoxicity. CD137 stimulation also restored the $CD8^+$ T cell response to the immunodominant influenza epitope in the absence of CD28 stimulation (29). These studies suggest that promoting $CD8^+$ T cell responses by modifying CD137 signaling may be a useful approach to improve antiviral $CD8^+$ T cell responses.

Alloresponses

CD137/CD137L interaction plays an important role in regulating alloresponses *in vivo*. Anti-CD137 mAbs enhance cardiac allograft and MHC-mismatched skin transplant rejection with dramatically increased INF-γ production by $CD8^+$ T cells and CTL activity against alloantigens (27). Blocking CD137/CD137L interaction by anti-CD137L mAbs significantly inhibited rejection of intestinal allografts by $CD8^+$ but not $CD4^+$ T cells (46). However, Blazar et al. proved that anti-CD137 mAbs promoted both $CD8^+$ and $CD4^+$ T cell-mediated graft-versus-host disease (GVHD) and host anti-donor-mediated graft rejection could be regulated through CD137/CD137L interaction by using anti-CD137 mAbs, $CD137^{-/-}$ donor T cells, or $CD137L^{-/-}$ recipients (22). These studies imply that blocking CD137/CD137L interaction may reduce GVHD and prevent $CD8^+$ T cell-mediated allograft rejection. For allograft rejection involving both $CD4^+$ and $CD8^+$ T cells, combined blockade of CD137/CD137L and other costimulatory signaling is required.

Autoimmune diseases

T cells are involved in the pathogenesis of many autoimmune diseases. The activation of T cells in response to their cognate peptide/MHC targets requires costimulatory signals delivered by APCs occurring at multiple steps (47). Conventional costimulation blockade is an attractive therapeutic approach for the treatment of T cell-dependent auto-

immune diseases. In the treatment of experimental and human autoimmune diseases, effort has been devoted to blocking several costimulatory pathways, such as CD28/B7, CD40L/CD40 and OX-40L/OX-40R, with either soluble receptors or neutralizing anti-ligand mAbs (48-51). However, recent studies found that costimulatory agonists of CD137 could also prevent and have therapeutic effects on $CD4^+$ T cell-involved autoimmune diseases (31-34).

We first found that a single low dose of agonistic anti-CD137 mAb (2A) treatment prevented the development of EAE, a Th1 cell-mediated demyelinating disease of the central nervous system used as a murine model for human multiple sclerosis (31). Draining lymph node cells from anti-4-1BB-treated mice failed to respond to antigen stimulation *in vitro* or to transfer disease to RAG-1-deficient recipient mice. When treatment was initiated after disease onset, early EAE relapse was also inhibited. Our studies supported the idea that agonistic anti-4-1BB mAbs treatment initially increased T cell activation, and then promoted the clearance of these activated $CD4^+$ T cells, resulting in the attenuation of their effector functions.

Furthermore, Foell et al. and wefound that administering agonistic anti-CD137 mAbs also showed promising therapeutic effect in both $CD4^+$ T cell and B cell involved spontaneous systemic autoimmune disease. Moreover, the two studies found similar results despite using different anti-CD137 mAb clones and different SLE animal models (32, 33). MRL/lpr mice spontaneously develop lymphadenopathy and a severe autoimmune disease resembling human SLE due to the lymphoproliferative (lpr) mutation in the *Fas* gene. We found that short-term treatment with anti-CD137 dramatically blocked lymphadenopathy and spontaneous autoimmune diseases in MRL/lpr mice, ultimately leading to their prolonged survival. More importantly, this therapeutic regimen was also effective when started after the mice had already showed clinically detectable autoimmune disease (32). The therapeutic effects of anti-CD137 were mediated by the depletion of auto-reactive B cells, activated $CD4^+$ T cells and the aberrant $CD4^-CD8^-B220^+$ $CD3^+$ T cells that principally contribute to lymphadenopathy in MRL/lpr mice. In accordance with our studies, Mittler's group found similar therapeutic effects when treating with another agonistic mAb against CD137 (3H3) in a different, but commonly used human SLE murine model: NZB×NZW F_1 mice (33). They found that giving lupus-prone NZB×NZW F_1 female mice three injections of anti-CD137 mAbs between 26 and 35 weeks of age reversed acute disease, blocked chronic disease, and prolonged the mice's lifespan. Autoantibody production in treated mice, regardless of their age or disease status, was rapidly suppressed without inducing systemic immunosuppression or massive depletion of lymphocytes. In this model,

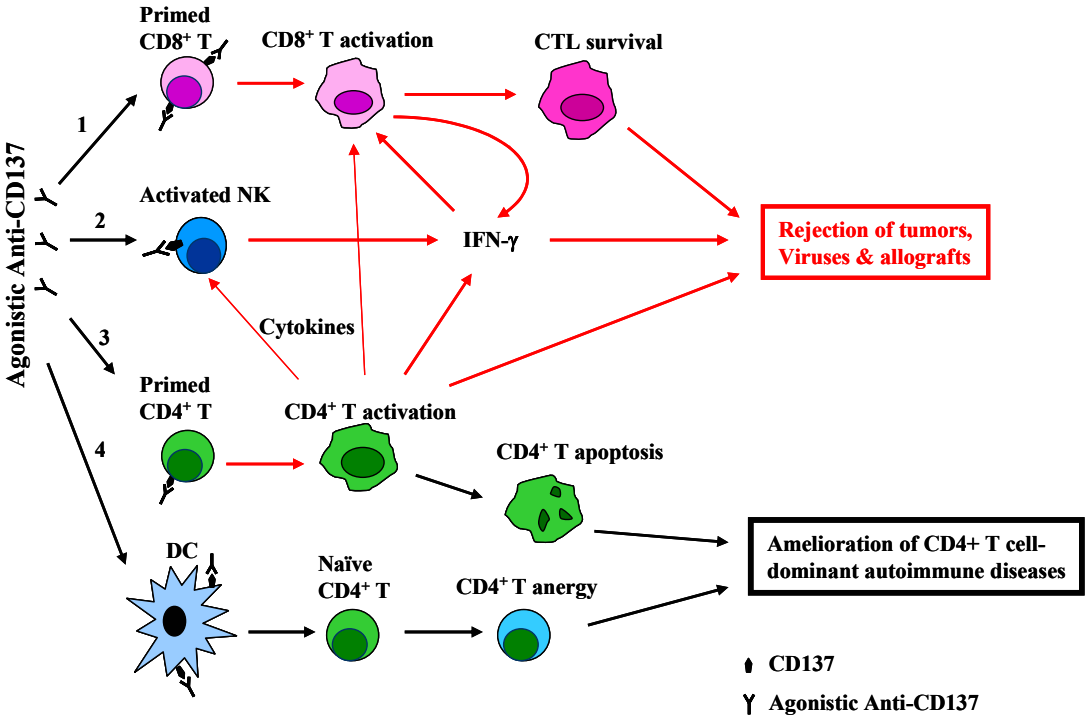

Figure 1. Diametric outcomes of anti-CD137 immunotherapy in disease models. CD137 costimualtion can either increase or inhibit immune responses *in vivo*, leading to tumor and allograft rejection, virus clearance and autoimmune disease amelioration. (1) Anti-CD137 treatment enhances primed $CD8^+$ T cell activation, proliferation and IFN-γ production; it also increases CTL activity and survival. These effects lead to tumor/virus/allograft rejection and augmented memory responses. They might also promote CD8-involved autoimmunity. (2) Anti-CD137 can also interact with activated NK cells to promote their proliferation and IFN-γ production which further modulates $CD8^+$ CTL activity. (3) Anti-CD137 treatment initially increases the activation of primed $CD4^+$ T cells which can provide cytokine help for $CD8^+$ T cell and NK cell activation and functional maturation, and subsequently promotes $CD4^+$ T cell apoptosis. (4) Agonist anti-CD137 interacts with dendritic cells (DCs) to prevent them from presenting antigens to or properly priming naïve $CD4^+$ T cells, leading to T cell anergy. Both $CD4^+$ T cell apoptosis and anergy contribute to amelioration of $CD4^+$ T cell-dominant autoimmune diseases.

adoptive transfer of antigen-primed $CD4^+$ T cells or DCs overrode anti-CD137-mediated protection, which suggests that unresponsiveness is not achieved by active suppression. The authors hypothesize that CD137-mediated signaling anergized $CD4^+$ T cells during priming at the DC interface.

However, CD137 engagement *in vivo* does not ameliorate all autoimmune diseases. Transgenic non-obese diabetic (NOD) mice overexpressing membrane-bound agonistic anti-CD137 scFv in pancreatic beta cells exhibited increased GAD-specific T cell responses, and developed more severe diabetes than their non-transgenic littermates, with earlier onset, faster diabetic processes, and higher mortality (52). Our preliminary data also showed that anti-CD137 treatment starting around the onset of disease promoted disease onset in NOD mice (Sun et al. unpublished data). Because CD137 costimulation always provides positive signaling to $CD8^+$ T cells and prolongs their survival, $CD8^+$ T cell-involved autoimmune diseases such as type I diabetes may not respond well to anti-CD137-based immunotherapy or may require combined treatment with CD8 response inhibitors. It is likely that anti-CD137 based immunotherapy be more effective in $CD4^+$ T cell-dominant autoimmune diseases.

Diametric outcomes of CD137 costimulation *in vivo*

The above studies showed that CD137 engagement by agonist mAbs *in vivo* can either augment or suppress an immune response depending on the type of immunocyte involved, cellular activation status, and the nature of the immune response (Figure 1). Most studies support the view that CD137 engagement preferentially costimulates $CD8^+$ T cells and prolongs their survival compared with $CD4^+$ cells (15, 27, 32). Deficiencies of CD137/CD137L interaction result in impaired $CD8^+$ T cell responses but normal $CD4^+$ T cell responses (23, 35, 53). While CD137 costimulation always promotes $CD8^+$ T cell-mediated immune responses *in vivo*, the nature of $CD4^+$ T cell responses remains controversial. One possible explanation for the divergent responses of $CD4^+$ and $CD8^+$ T cells to CD137 cross-linking *in vivo* is that CD137 signaling promotes the activation of both $CD4^+$ and $CD8^+$ T cells, but provides survival signaling only for $CD8^+$ T cells and causes activation induced cell death (AICD) of $CD4^+$ T cells. In the tumor and virus models, the therapeutic effect of anti-CD137 is largely dependent on $CD8^+$ T cells and IFN-γ (28, 29, 36-39, 43). Anti-CD137 treatment enhances $CD8^+$ T cell proliferation and IFN-γ production, increases CTL activity and prolongs their lifespan, which lead to tumor/virus/allograft rejection and augmented memory responses. Anti-CD137 could also interact with activated NK cells to promote their proliferation and IFN-γ production which further modulates $CD8^+$ CTL activity (3, 26).

Our studies supported dual phase effects of CD137 costimulation on $CD4^+$ T cells *in vivo*. It initially increased the activation of $CD4^+$ T cells, and subsequently attenuated their effector functions by promoting their clearance by apoptosis (31). The early stage activation of $CD4^+$ T cells could be sufficient to provide cytokine help for $CD8^+$ T cell and NK cell activation and functional maturation. For an allogenic T cell response, due to a high proportion of T cell repertoires involved and relatively acute responses, CD137 signaling-activated $CD4^+$ T cells may mediate rejection before the down-regulation of their functions. However, given the relatively slow process of autoimmune disease, autoreactive $CD4^+$ T cells might undergo AICD before they induce strong enough downstream events to cause severe tissue damage or death. The mechanism involved in CD137 stimulation-induced AICD of $CD4^+$ T cell is not clear and may be indirect.

Another possible explanation for CD137-mediated suppression of $CD4^+$ T cell function is that CD137 signaling induces $CD4^+$ T cell anergy during priming at the DC interface (30, 33). Timing may be important; when agonistic anti-CD137 mAbs are administered before T cells get properly primed, they will stimulate DCs and prevent antigen presentation to naïve T cells, leading to T cell anergy. However, once the T cells are primed, CD137 agonists will increase T cell proliferation and functional maturation.

Regulatory cells may also play a role in CD137-signaling-mediated immunosuppression. It has been shown that Toll-like receptor ligands and CD137 costimulation activated $CD8^+$ T cells can inhibit $CD4^+$ T cell responses (54). Although CD137 is constitutively expressed on $CD4^+CD25^+$ regulatory T cells (5, 6), there is no direct evidence to show that CD137 signaling plays a role in modifying regulatory T cell function.

CD137 costimulation suppresses T cell-dependent humoral immune response and autoantibody production in several ways. First, it inhibits helper T cell function by either anergy or depletion (30, 32, 33). Second, it can also induce depletion of B cells (32). In support of this, transgenic mice expressing CD137L on class II positive cells show selective depletion of B cells, low levels of circulating IgG and defective humoral responses to antigen challenge (55). Third, CD137 costimulation induces a T cell-dependent down-regulation of follicular dendritic cells (FDCs), which are the antigen-trapping accessory cells of the germinal centers (GCs) and are essential for the development of humoral immune responses and memory (Sun et al. unpublished data). The role of CD137 in humoral immune response requires further investigation.

In conclusion, CD137 signaling can either increase or inhibit immune responses *in vivo*, leading to tumor rejection, virus clearance and autoimmune disease amelioration. Depending on the stage of T cell responses and the type of inflammatory environment involved, we can target CD137 by pharmacologic ligation to enhance or curtail an ongoing immune response. Because this ligation appears to produce dual outcomes, caution must be taken with this approach due to the strong possibility of side effects. The therapeutic regimen must be based on a clear understanding of the pathogenesis, pathogenic cell types involved in the target disease, and the health situation of the patients selected; targeting CD137 in patients with multiple health problems might ameliorate one disease while making another worse. However, when used wisely, CD137 cross-linking may provide an effective new therapeutic avenue in the treatment of several diseases.

Acknowledgement

We thank Dr. Lieping Chen for suggestions and reagents. Y.S. is the recipient of a NIH training fellowship (T32

HL07237). The work is in part supported by NIH grants.

References

1. Pollok KE, Kim YJ, Zhou Z, et al. Inducible T cell antigen 4-1BB. Analysis of expression and function. J Immunol. 1993; 150:771-781.
2. Kwon BS, Weissman SM. cDNA sequences of two inducible T-cell genes. Proc Natl Acad Sci U S A. 1989; 86:1963-1967.
3. Melero I, Johnston JV, Shufford WW, Mittler RS, Chen L. NK1.1 cells express 4-1BB (CDw137) costimulatory molecule and are required for tumor immunity elicited by anti-4-1BB monoclonal antibodies. Cell Immunol. 1998;190:167-172.
4. Kwon B, Moon CH, Kang S, Seo SK, Kwon BS. 4-1BB: still in the midst of darkness. Mol Cells. 2000;10:119-126.
5. Gavin MA, Clarke SR, Negrou E, Gallegos A, Rudensky A. Homeostasis and anergy of CD4+CD25+ suppressor T cells in vivo. Nat Immunol. 2002;3:33-41.
6. McHugh RS, Whitters MJ, Piccirillo CA, et al. CD4(+)CD25 (+) immunoregulatory T cells: gene expression analysis reveals a functional role for the glucocorticoid-induced TNF receptor. Immunity. 2002;16:311-323.
7. Kwon BS, Tan KB, Ni J, et al. A newly identified member of the tumor necrosis factor receptor superfamily with a wide tissue distribution and involvement in lymphocyte activation. J Biol Chem. 1997;272:14272-14276.
8. Heinisch IV, Daigle I, Knopfli B, Simon HU. CD137 activation abrogates granulocyte-macrophage colony stimulating factor-mediated anti-apoptosis in neutrophils. Eur J Immunol. 2000; 30:3441-3446.
9. Futagawa T, Akiba H, Kodama T, et al. Expression and function of 4-1BB and 4-1BB ligand on murine dendritic cells. Int Immunol. 2002;14:275-286.
10. Wilcox RA, Chapoval AI, Gorski KS, et al. Cutting edge: Expression of functional CD137 receptor by dendritic cells. J Immunol. 2002;168:4262-4267.
11. Goodwin RG, Din WS, Davis-Smith T, et al. Molecular cloning of a ligand for the inducible T cell gene 4-1BB: a member of an emerging family of cytokines with homology to tumor necrosis factor. Eur J Immunol. 1993;23:2631-2641.
12. Pollok KE, Kim YJ, Hurtado J, Zhou Z, Kim KK, Kwon BS. 4-1BB T-cell antigen binds to mature B cells and macrophages, and costimulates anti-mu-primed splenic B cells. Eur J Immunol. 1994;24:367-374.
13. Alderson MR, Smith CA, Tough TW, et al. Molecular and biological characterization of human 4-1BB and its ligand. Eur J Immunol. 1994;24:2219-2227.
14. Hurtado JC, Kim YJ, Kwon BS. Signals through 4-1BB are costimulatory to previously activated splenic T cells and inhibit activation-induced cell death. J Immunol. 1997;158: 2600-2609.
15. Takahashi C, Mittler RS, Vella AT. Cutting edge: 4-1BB is a bona fide CD8 T cell survival signal. J Immunol. 1999;162: 5037-5040.
16. Saoulli K, Lee SY, Cannons JL, et al. CD28-independent, TRAF2-dependent costimulation of resting T cells by 4-1BB ligand. J Exp Med. 1998;187:1849-1862.
17. Cannons JL, Choi Y, Watts TH. Role of TNF receptor-associated factor 2 and p38 mitogen-activated protein kinase activation during 4-1BB-dependent immune response. J Immunol. 2000;165:6193-6204.
18. Cannons JL, Hoeflich KP, Woodgett JR, Watts TH. Role of the stress kinase pathway in signaling *via* the T cell costimulatory receptor 4-1BB. J Immunol. 1999;163:2990-2998.
19. Lee HW, Park SJ, Choi BK, Kim HH, Nam KO, Kwon BS. 4-1BB promotes the survival of $CD8^+$ T lymphocytes by increasing expression of Bcl-XL and Bfl-1. J Immunol. 2002; 169:4882-4888.
20. Cooper D, Bansal-Pakala P, Croft M. 4-1BB (CD137) controls the clonal expansion and survival of CD8 T cells in vivo but does not contribute to the development of cytotoxicity. Eur J Immunol. 2002;32:521-529.
21. Lee HW, Nam KO, Park SJ, Kwon BS. 4-1BB enhances CD8+ T cell expansion by regulating cell cycle progression through changes in expression of cyclins D and E and cyclin-dependent kinase inhibitor p27kip1. Eur J Immunol. 2003;33: 2133-2141.
22. Blazar BR, Kwon BS, Panoskaltsis-Mortari A, Kwak KB, Peschon JJ, Taylor PA. Ligation of 4-1BB (CDw137) regulates graft-versus-host disease, graft-versus-leukemia, and graft rejection in allogeneic bone marrow transplant recipients. J Immunol. 2001;166:3174-3183.
23. Tan JT, Whitmire JK, Ahmed R, Pearson TC, Larsen CP. 4-1BB ligand, a member of the TNF family, is important for the generation of antiviral CD8 T cell responses. J Immunol. 1999;163:4859-4868.
24. DeBenedette MA, Wen T, Bachmann MF, et al. Analysis of 4-1BB ligand (4-1BBL)-deficient mice and of mice lacking both 4-1BBL and CD28 reveals a role for 4-1BBL in skin allograft rejection and in the cytotoxic T cell response to influenza virus. J Immunol. 1999;163:4833-4841.
25. Tan JT, Whitmire JK, Murali-Krishna K, et al. 4-1BB costimulation is required for protective anti-viral immunity after peptide vaccination. J Immunol. 2000;164:2320-2325.
26. Wilcox RA, Tamada K, Strome SE, Chen L. Signaling through NK cell-associated CD137 promotes both helper function for $CD8^+$ cytolytic T cells and responsiveness to IL-2 but not cytolytic activity. J Immunol. 2002;169:4230-4236.
27. Shuford WW, Klussman K, Tritchler DD, et al. 4-1BB costimulatory signals preferentially induce $CD8^+$ T cell proliferation and lead to the amplification in vivo of cytotoxic T cell responses. J Exp Med. 1997;186:47-55.
28. Melero I, Shuford WW, Newby SA, et al. Monoclonal antibodies against the 4-1BB T-cell activation molecule eradicate established tumors. Nat Med. 1997;3:682-685.
29. Halstead ES, Mueller YM, Altman JD, Katsikis PD. In vivo stimulation of CD137 broadens primary antiviral CD8+ T cell responses. Nat Immunol. 2002;3:536-541.
30. Mittler RS, Bailey TS, Klussman K, Trailsmith MD, Hoffmann MK. Anti-4-1BB monoclonal antibodies abrogate T cell-dependent humoral immune responses in vivo through the induction of helper T cell anergy. J Exp Med. 1999;190: 1535-1540.
31. Sun Y, Lin X, Chen HM, et al. Administration of agonistic anti-4-1BB monoclonal antibody leads to the amelioration of experimental autoimmune encephalomyelitis. J Immunol. 2002;168:1457-1465.
32. Sun Y, Chen HM, Subudhi SK, et al. Costimulatory molecule-targeted antibody therapy of a spontaneous autoimmune disease. Nat Med. 2002;8:1405-1413.
33. Foell J, Strahotin S, O'Neil SP, et al. CD137 costimulatory T cell receptor engagement reverses acute disease in lupus-prone NZB x NZW F1 mice. J Clin Invest. 2003;111:1505-1518.
34. Sun Y, Subudhi SK, Fu YX. Co-stimulation agonists as a new immunotherapy for autoimmune diseases. Trends Mol Med. 2003;9:483-489.
35. Kwon BS, Hurtado JC, Lee ZH, et al. Immune responses in 4-1BB (CD137)-deficient mice. J Immunol. 2002;168:5483-5490.
36. Wilcox RA, Flies DB, Zhu G, et al. Provision of antigen and CD137 signaling breaks immunological ignorance, promoting regression of poorly immunogenic tumors. J Clin Invest. 2002; 109:651-659.
37. Miller RE, Jones J, Le T, et al. 4-1BB-specific monoclonal antibody promotes the generation of tumor-specific immune responses by direct activation of CD8 T cells in a CD40-dependent manner. J Immunol. 2002;169:1792-1800.
38. Kim JA, Averbook BJ, Chambers K, et al. Divergent effects of 4-1BB antibodies on antitumor immunity and on tumor-reactive T-cell generation. Cancer Res. 2001;61:2031-2037.

39. Strome SE, Martin B, Flies D, et al. Enhanced therapeutic potential of adoptive immunotherapy by in vitro CD28/4-1BB costimulation of tumor-reactive T cells against a poorly immunogenic, major histocompatibility complex class I-negative A9P melanoma. J Immunother. 2000;23:430-437.
40. May KF, Jr., Chen L, Zheng P, Liu Y. Anti-4-1BB monoclonal antibody enhances rejection of large tumor burden by promoting survival but not clonal expansion of tumor-specific $CD8^+$ T cells. Cancer Res. 2002;62:3459-3465.
41. Chen SH, Pham-Nguyen KB, Martinet O, et al. Rejection of disseminated metastases of colon carcinoma by synergism of IL-12 gene therapy and 4-1BB costimulation. Mol Ther. 2000; 2:39-46.
42. Ye Z, Hellstrom I, Hayden-Ledbetter M, Dahlin A, Ledbetter JA, Hellstrom KE. Gene therapy for cancer using single-chain Fv fragments specific for 4-1BB. Nat Med. 2002;8:343-348.
43. Wilcox RA, Flies DB, Wang H, et al. Impaired infiltration of tumor-specific cytolytic T cells in the absence of interferon-gamma despite their normal maturation in lymphoid organs during CD137 monoclonal antibody therapy. Cancer Res. 2002;62:4413-4418.
44. Wilcox RA, Tamada K, Flies DB, et al. Ligation of CD137 receptor prevents and reverses established anergy of $CD8^+$ cytolytic T lymphocytes in vivo. Blood. 2004;103:177-184.
45. Bertram EM, Lau P, Watts TH. Temporal segregation of 4-1BB versus CD28-mediated costimulation: 4-1BB ligand influences T cell numbers late in the primary response and regulates the size of the T cell memory response following influenza infection. J Immunol. 2002;168:3777-3785.
46. Wang J, Guo Z, Dong Y, et al. Role of 4-1BB in allograft rejection mediated by $CD8^+$ T cells. Am J Transplant. 2003; 3:543-551.
47. Lanzavecchia A, Sallusto F. Dynamics of T lymphocyte responses: intermediates, effectors, and memory cells. Science. 2000;290:92-97.
48. Salomon B, Bluestone JA. Complexities of CD28/B7: CTLA-4 costimulatory pathways in autoimmunity and transplantation. Annu Rev Immunol. 2001;19:225-252.
49. Weinberg AD, Wegmann KW, Funatake C, Whitham RH. Blocking OX-40/OX-40 ligand interaction *in vitro* and *in vivo* leads to decreased T cell function and amelioration of experimental allergic encephalomyelitis. J Immunol. 1999;162: 1818-1826.
50. Nohara C, Akiba H, Nakajima A, et al. Amelioration of experimental autoimmune encephalomyelitis with anti-OX40 ligand monoclonal antibody: a critical role for OX40 ligand in migration, but not development, of pathogenic T cells. J Immunol. 2001;166:2108-2115.
51. Yoshioka T, Nakajima A, Akiba H, et al. Contribution of OX40/OX40 ligand interaction to the pathogenesis of rheumatoid arthritis. Eur J Immunol. 2000;30:2815-2823.
52. Sytwu HK, Lin WD, Roffler SR, et al. Anti-4-1BB-based immunotherapy for autoimmune diabetes: lessons from a transgenic non-obese diabetic (NOD) model. J Autoimmun. 2003;21:247-254.
53. Tan JT, Ha J, Cho HR, et al. Analysis of expression and function of the costimulatory molecule 4-1BB in alloimmune responses. Transplantation. 2000;70:175-183.
54. Myers L, Takahashi C, Mittler RS, Rossi RJ, Vella AT. Effector CD8 T cells possess suppressor function after 4-1BB and Toll-like receptor triggering. Proc Natl Acad Sci U S A. 2003; 100:5348-5353.
55. Zhu G, Flies DB, Tamada K, et al. Progressive depletion of peripheral B lymphocytes in 4-1BB (CD137) ligand/I-Ealpha-transgenic mice. J Immunol. 2001;167:2671-2676.