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Functional Dichotomy between OX40 and 4-1BB in Modulating Effector CD8 T Cell Responses

Seung-Woo Lee,* Yunji Park,† Aihua Song,* Hilde Cheroutre,† Byoung S. Kwon,‡ and Michael Croft2*

Members of the TNFR family are thought to deliver costimulatory signals to T cells and modulate their function and survival. In this study, we compare the role of two closely related TNFR family molecules, OX40 and 4-1BB, in generating effector CD8 T cells to Ag delivered by adenovirus. OX40 and 4-1BB were both induced on responding naive CD8 T cells, but 4-1BB exhibited faster and more sustained kinetics than OX40. OX40-deficient CD8 T cells initially expanded normally; however, their accumulation and survival at late times in the primary response was significantly impaired. In contrast, 4-1BB-deficient CD8 T cells displayed hyperresponsiveness, expanding more than wild-type cells. The 4-1BB-deficient CD8 T cells also showed enhanced maturation attributes, whereas OX40-deficient CD8 T cells had multiple defects in the expression of effector cell surface markers, the synthesis of cytokines, and in cytotoxic activity. These results suggest that, in contrast to current ideas, OX40 and 4-1BB can have a clear functional dichotomy in modulating effector CD8 T cell responses. OX40 can positively regulate effector function and late accumulation/survival, whereas 4-1BB can initially operate in a negative manner to limit primary CD8 responses. The Journal of Immunology, 2006, 177: 4464–4472.

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42), granulocytes (43), and mast cells (44). Thus, various targets of 4-1BB action are possible, including regulatory APC or T cells, but pathogenic T cells have not been ruled out as recipients of suppressive 4-1BB signals, raising the issue of whether 4-1BB can have dual roles on T cells, both positive and negative. In this study, we have addressed the specific role of OX40 and 4-1BB when expressed on responding naive or primed CD8 T cells. To rule out possible effects from other immune cells, we adoptively transferred OT-I TCR-transgenic CD8 T cells from mice deficient in OX40 or 4-1BB into wild-type congenic mice, and then infected them with recombinant adenovirus expressing a membrane-bound form of the Ag OVA (Ad-mOVA) that is recognized by the T cells. Significantly, we show that OX40 and 4-1BB regulate effector CD8 T cell responses in a very opposing fashion. OX40 is important to maintain CD8 T cell numbers and functionality at the peak of primary responses, but 4-1BB acts in an inhibitory manner suppressing CD8 T cell expansion and function.

Materials and Methods

Mice and virus

OT-I TCR-transgenic mice bred on the C57Bl/6 background were used as a source of SIINFEKL peptide-reactive CD8 T cells. OX40+/+ and 4-1BB−/− mice were bred with OT-I mice to generate gene-deficient OT-I mice. C57BL/6, B6.PL-Thy1.1 (Thy1.1+), and B6.SJL-Ptprca Pep3b<sup>B</sup>Biy1 (CD45.1+) mice were purchased from The Jackson Laboratory. Ad-mOVA was purchased from Gene Transfer Vector Core, University of Iowa (Iowa City, IA).

Adoptive transfer of CD8 T cells and virus challenge

Naive CD8 T cells were purified from pooled lymph node (LN) and spleen cells of wild-type and gene-deficient OT-I mice as previously described (45). Briefly, total CD8 T cells were purified first with CD8 T cell isolation kits (Miltenyi Biotec) following the manufacturer’s protocol. Naive CD8 T cells were purified from total CD8 T cells through MACS by incubation with pretitrated biotin labeled anti-CD44 followed by antibiotin micro-bead. Purified naive CD8 T cells were phenotypically (CD44<sup>lo</sup>CD62L<sup>hi</sup>) mice were injected i.v. into congenic B6.Ptprca<sup>B</sup> mice and 2 days after Ad-mOVA (2 x 10<sup>7</sup> PFU) was injected i.m. into both quadriceps muscles. In some cases, purified naive CD8 T cells were labeled with 5 μM CFSE (Molecular Probes) for tracking cell division before adoptive transfer (~1–2 x 10<sup>6</sup> cells). For secondary responses of CD8 T cells, OT-I cells were stimulated with SIINFEKL peptide for 3 days and then rested with IL-15 (PeproTech) for another 3 days as previously described (22). Primed OT-I cells (5–10<sup>5</sup> cells) were adoptively transferred into congenic mice and infected i.m. with Ad-mOVA (2 x 10<sup>7</sup> PFU).

Flow cytometry analysis

Recipient mice were sacrificed at the indicated times, and the draining LN and spleen were harvested. An aliquot of cells was stained with anti-CD8 PerCP and anti-Thy1.2 FITC (BD Pharmingen). For staining of cell surface markers, the following Abs were used: PE-labeled anti-CD44, anti-CD69, anti-CD45.1, anti-CD27, anti-CD122, anti-CD62L, anti-CD43 (1B1), anti-CD25, or biotin-labeled anti-OX40 (all from BD Pharmingen), anti-4-1BB (Biolegend) followed by PE-labeled streptavidin (Molecular Probes). Intracellular staining was performed as previously described. Cells were stained with anti-CD8 PerCP and anti-Thy1.2 FITC. Cells then were fixed and permeabilized followed by intracellular staining with anti-TNF PE, anti-IFN-γ, and allogenic cytokinin (BD Pharmingen), anti-human granamne B PE (CalUg Laboratories), anti-mouse Bcl-2 PE, or PE-labeled isotype control Abs (BD Pharmingen).

CTL assay

Spleen cells were harvested from infected mice at day 6 and the number of activated OT-I cells (CD8<sup>+</sup>Thy1.2<sup>+</sup>CD44<sup>hi</sup>) in individual spleens were normalized by FACS. Target cells were prepared using total spleen cells from CD45.1<sup>+</sup> congenic mice that were differentially labeled with CFSE as previously described (46). Abs were purchased with OVA<sub>123-139</sub> (20 μM) to serve as the internal control. Cells were 1:1 mixed and then cocultured with spleen cells from infected mice for 7 h. Specific cytotoxicity was calculated by determining the ratio of CFSE<sup>low</sup>/CFSE<sup>high</sup> by FACS. For CTL assay at day 10, total CD8 T cells were purified first with MACS and then incubated with target cells for 18 h.

Results

Overlapping and distinct expression profiles of OX40 and 4-1BB on CD8 T cells during priming

OX40 and 4-1BB have been thought to have similar patterns of expression on activated T cells, largely based on results from in vitro culture systems. Both molecules show clear inducible expression, which starts several hours after naive T cell activation and usually peaks 2–4 days into the response (4). However, expression patterns of both molecules have not been characterized well in vivo. After intranasal challenge with influenza virus, a minor population of T cells infiltrating the lung expressed OX40, but 4-1BB was absent (20). In other studies, CD8 T cells were shown to minimally express OX40 after s.c. injection of high dose OVA with LPS and 4-1BB was expressed only transiently during the initial 12–36 h (27). In this study, we first compared expression of both molecules on responding naive CD8 cells after i.m. infection with Ad-mOVA in draining LN (Fig. 1A) and spleen (Fig. 1B). To aid visualizing expression throughout the course of cell division, we adoptively transferred a relatively high number of CFSE-labeled OT-I transgenic CD8 T cells (1–2 x 10<sup>5</sup>) into congenic mice.

The 4-1BB was detected at day 1 after infection before the CD8 population had divided. The majority of CD8 cells expressed 4-1BB between days 2 and 3, and then expression declined gradually thereafter, with levels being similar regardless of the number of divisions undergone. Interestingly, 4-1BB was long lasting on a significant population of responding CD8 cells and still expressed between days 4 and 7. In contrast, OX40 was largely absent at day 1 after infection and not expressed on activated but undivided cells. Corresponding with presumed Ag load, OX40 was induced more on LN compared with spleen CD8 cells between days 2 and 3. Interestingly, the level of OX40 correlated with the number of cell divisions, being higher in CD8 cells that had undergone more divisions. However, it sharply declined after day 3 and became undetectable on most responding CD8 cells, although a small population of cells (~5%) that had undergone more than eight divisions still expressed low levels of OX40 at late times. In general, OX40<sup>−/−</sup> and 4-1BB<sup>−/−</sup> CD8 cells showed wild-type cell division profiles and expressed the reciprocal molecule comparably to wild-type CD8 cells (Fig. 1C), suggesting that each molecule has no apparent role for early cell division and does not cross-regulate the expression of the other. Responsiveness of 4-1BB- and OX40-deficient CD8 cells to PMA/ionomycin or anti-CD3 in APC-free systems in vitro was also comparable to wild-type cells, also implying the absence of developmental defects in the CD8 cells from the knockout animals. Collectively, these results show that OX40 and 4-1BB have very distinct expression patterns on activated CD8 T cells responding to adenovirus. Both molecules can be coexpressed at a particular time during priming, but 4-1BB is expressed earlier than OX40, and 4-1BB is maintained over time, whereas OX40 is more transient.

Opposing roles of OX40 and 4-1BB in regulating the accumulation of CD8 T cells

To understand the functional role of OX40 and 4-1BB in priming of CD8 T cells, we adoptively transferred a lower number (1 x 10<sup>5</sup>) of naive (>99% of CD44<sup>hi</sup>CD62L<sup>hi</sup>) wild-type or gene-deficient OT-I CD8 cells into wild-type congenic recipients and then tracked them after infection with Ad-mOVA. Initial expansion of CD8 cells was seen from day 3, peaking at days 5–7, and...
then the cells underwent a sharp contraction by day 10 (Fig. 2). Surprisingly, 4-1BB−/− CD8 cells showed clear hyperresponsiveness. The expansion of 4-1BB−/− cells started to outpace that of wild-type cells at day 3 (average number, OT-I cells 3.58 × 10^4 vs 4-1BB−/− OT-I cells 7.23 × 10^4), and this result was strongly evident by day 5 when ~2-fold more CD8 cells were found (Fig. 2A). Enhanced numbers of 4-1BB-deficient cells were visualized through to day 7; however, there was no statistical difference between wild-type and 4-1BB−/− CD8 cells at this time due to the individual variation among mice. Most likely some CD8 cells lacking 4-1BB already started to undergo clonal contraction while others were still expanding at this time point, thus accounting for the high variability. These results suggest that OX40 and 4-1BB have opposing roles in regulating clonal expansion of CD8 T cells in the primary response to adenovirus.

Because we found differences in the accumulation of CD8 cells (Fig. 2), we examined in vivo apoptosis (annexin V) and the expression of the antiapoptotic molecule Bcl-2 by flow cytometry at days 5–7. However, we could not find significant differences in the absence of OX40 or 4-1BB (data not shown).

**Altered differentiation of effector CD8 T cells in the absence of OX40 or 4-1BB**

When naive CD8 T cells differentiate into fully functional effectors, a number of phenotypic changes occur (47–49). To determine the role of OX40 and 4-1BB in the differentiation process we characterized effector markers at days 5–7 after infection. At the peak of response, wild-type and OX40/4-1BB-deficient effector CD8 cells were CD69<sup>−</sup>, CD25<sup>−</sup>, CD44<sup>high</sup>, and CD122<sup>high</sup> (data not shown). We did not find any significant difference in these markers in the absence of OX40 or 4-1BB (data not shown). Additionally, wild-type and OX40/4-1BB-deficient effector cells expressed a comparable level of activation-associated glycoform of murine CD43 (50) and completely down-regulated CD127 (IL-7R) at day 5, with some cells regaining expression at day 7 as previously reported (51) (Fig. 3). These results suggest that OX40<sup>−/−</sup> and 4-1BB<sup>−/−</sup> CD8 cells gained a number of characteristics of differentiated effectors. However, we found differences in the expression pattern of CD27 and CD62L,
two other differentiation markers (Fig. 3). OX40−/− CD8 cells exhibited less down-regulation of both molecules than wild-type cells at day 5 and day 7. In contrast, 4-1BB−/− CD8 cells exhibited increased kinetics of down-regulation. Based on previous reports showing that highly activated effector CD8 T cells down-regulate CD62L (49, 51), and that CD27 loss is also a characteristic of maturation (52, 53), these results suggested that OX40 was required for full differentiation, whereas 4-1BB acted to limit differentiation at an early time of priming.

Defective functional activity of effector CD8 T cells in the absence of OX40

To address any role of OX40 and 4-1BB in regulating effector function of CD8 T cells, we examined the ability to secrete IFN-γ and TNF. A large proportion of wild-type and 4-1BB−/− CD8 cells rapidly secreted both cytokines by day 6 after infection, as measured by intracellular staining after in vitro stimulation with SIINFEKL peptide (Fig. 4, A and B). However, far fewer OX40−/− CD8 cells were positive for both cytokines. Interestingly at this time, OX40−/− CD8 cells synthesized IFN-γ almost equivalently, but showed a strong defect in the ability to make TNF (Fig. 4B). Effector cytokine production by all CD8 populations diminished over time, but a substantial number of wild-type and 4-1BB−/− CD8 cells still produced both cytokines after restimulation at day 10 (Fig. 4C). Conversely, OX40−/− CD8 cells at this time showed impaired IFN-γ production as well as TNF production.

Lastly, we performed ex vivo direct CTL assays to test the killing activity of surviving CD8 cells. OT-I cells isolated at day 6 showed substantial cytotoxic activities (Fig. 5, A and B). Significantly, even after normalization of OT-I E:T ratios, OX40−/− CD8 cells showed a decreased cytotoxic activity, ~5:1 (E:T = 1.33:1) to 2-fold (E:T = 4:1) lower than that of wild-type CD8 cells (Fig. 5A). In contrast, 4-1BB−/− CD8 cells showed moderately higher cytotoxic activities. Similar results were found 8 days after infection (data not shown). Correlating with the altered cytotoxic activity of OX40-deficient T cells, we found that the majority of wild-type and 4-1BB−/− CD8 cells expressed high levels of granzyme B at day 6, whereas OX40−/− CD8 cells were strongly impaired in granzyme B expression (Fig. 5C). The level of granzyme B declined by day 8 in all CD8 cells, but there was still a clear defect in OX40−/− CD8 cells (Fig. 5D). Collectively, these results demonstrate that OX40 can control the killing activity of effector CD8 T cells through the regulation of cytotoxic genes such as granzyme B.

Enhanced secondary responsiveness of CD8 cells in the absence of 4-1BB

To further address the roles of OX40 and 4-1BB, we analyzed secondary responses of previously primed CD8 T cells. OT-I cells were stimulated in vitro and then rested in IL-15 to generate primed CD8 populations. These were then tracked after adoptive transfer, following an in vivo Ad-mOVA challenge. The majority of CD8 cells expressed OX40 and 4-1BB after 3 days during this secondary response (Fig. 6A). Primed 4-1BB−/− CD8 cells accumulated ~3-fold more than wild-type cells at day 5 after infection,
**FIGURE 4.** OX40 regulates cytokine production from effector CD8 T cells. Mice received wild-type and gene-deficient OT-I cells and were immunized as in Fig. 1. Pooled lymphoid cells from groups of four mice were restimulated with SIINFEKL peptide for 6 h, and CD8^"Thy1.2"^ cells were stained for intracellular IFN-γ and TNF at day 6 (A and B) and day 10 (C). B and C. The population expressing cytokines (circles) compared with an isotype control is shown. Data are representative of three experiments.
whereas primed OX40−/− CD8 cells showed a defect of expansion (Fig. 6B), largely analogous to the phenotype seen during the primary response of CD8 cells. Consistent with this finding, 4-1BB−/− CD8 cells showed more differentiated phenotypic and functional attributes, such as greater down-regulation of CD62L and increased IFN-γ and granzyme B expression (Fig. 6C). In contrast, no differences were observed between wild-type and OX40−/− CD8 cells. These results additionally illustrate that OX40 and 4-1BB play opposing roles in expansion and reactivity of CD8 cells.

Discussion

In this study we report that there is a functional divergence between OX40 and 4-1BB in modulating CD8 priming and effector CD8 responses to adenovirus-expressed Ag. In line with recent reports, OX40−/− CD8 T cells exhibited a defect in accumulating at high numbers over time, and importantly OX40 positively regulated the effector function of CD8 T cells. Surprisingly, in contrast to OX40, 4-1BB negatively regulated the expansion and maturation of effector CD8 T cells.

There have been inconsistencies reported in the literature regarding a role for OX40 in CD8 T cell responses. Agonist Abs to OX40 have shown clear positive effects on CD8 T cell responses in various models, suggesting CD8 cells can respond directly to OX40 signals (15, 16, 54). However, in such studies the target cell for these Abs has not been clear, and at most, only CD4 cells have been ruled out, still leaving the possibility that OX40 action on non-CD8 cells could indirectly augment CD8 priming. In contrast, our present data with OX40−/− CD8 cells responding to Ag provided by adenovirus, and our recent published data with response to Ag in CFA (21) or Ag expressed by a tumor (22), clearly establish that OX40 can control CD8 priming. However, other reports with OX40− or OX40L-deficient animals conversely have shown no role for these molecules in primary CD8 responses (17, 18, 27), begging the question of why OX40 sometimes can be relevant and sometimes irrelevant to CD8 priming. One factor to account for the discrepancies could be alternate expression patterns of OX40 under different physiological situations. OX40 is probably highly regulated on CD8 cells. OX40 was expressed only very transiently on CD8 cells at days 2–3 after adenoviral infection, in contrast to 4-1BB, which was expressed rapidly and for a long time. This transient OX40 expression correlates with that previously seen in a CFA inflammatory environment and in a tumor environment (P. Bansal-Pakala, A. Song, and M. Croft, unpublished observations). Most likely OX40 induction depends upon the amount of Ag as well as environmental factors such as cytokines and TLR signals made available by local inflammation. For example, in one study CD8 cells failed to express OX40 after injection of OVA in LPS, whereas 4-1BB was expressed (27), correlating with the functional conclusions from these authors that OX40 played no role in CD8 priming. Further studies will be required to understand how OX40 expression is regulated and what inflammatory stimuli control its expression.

In our present, and previous studies, OX40 does not appear to control initial priming events and early responses of CD8 T cells because naïve OX40−/− CD8 cells become activated and expand relatively normally. However, they failed to sustain their numbers over time, suggesting that in the absence of OX40 there is a defect in long-term division or survival. These results correspond with previous reports of OX40−/− CD4 and CD8 T cells that showed
OX40 suppressed apoptosis through up-regulation of Bcl-2 family proteins (10, 22). In this study, we could not detect any significant differences in the expression of Bcl-2 or in vivo apoptosis between wild-type and OX40−/− OT-1 cells, but this may be due to technical difficulties to assess these parameters in vivo (51) rather than reflective of an alternate mechanism of OX40 action.

As opposed to our previous studies of OX40 on CD8 cells in adjuvant and tumor systems, we show in response to adenovirus that OX40 can dramatically regulate differentiation and effector function. Again, it is not clear why OX40 has multiple alternate adjuvant and tumor systems, we show in response to adenovirus reflective of an alternate mechanism of OX40 action.

nical difficulties to assess these parameters in vivo (51) rather than (CD62LlowCD27low) after LCMV infection (49). Consistently, when we restimulated OT-1 cells in vitro we found that OX40−/− cells were not efficient in producing effector cytokines. OX40−/− CD8 cells showed a more pronounced defect in producing TNF compared with IFN-γ, indicating that the OX40 signal may differentially target this cytokine in fully activated CD8 T cells. Of note, a previous report showed that blocking OX40L with OX40-Ig reduced TNF production from CD8 T cells after influenza virus infection (55), and more recently Ito et al. (56) showed that OX40 signaling selectively promoted TNF secretion in human CD4 T cells in vitro. Thus, the regulation of TNF synthesis by OX40 may be common to CD4 and CD8 T cells in various inflammatory circumstances. As well as TNF production, another striking functional impairment in the absence of OX40 was decreased cytotoxicity. Interestingly, when costing activation markers and cytokines with granzyme B, we found that wild-type CD8 cells expressing high levels of granzyme B were phenotypically CD62Llow, CD27low, and TNF− (data not shown), and this group was the phenotype not generated when OX40 was absent. OX40 may then help to program genetic remodeling during the transition from naive to effector cells, resulting in the expression of signature genes central to effector CD8 T cells (57). However, preprimed OX40−/− CD8 cells showed functional characteristics comparable to those of wild-type cells in a secondary response, even though cell accumulation was decreased, possibly suggesting that a role in differentiation only applies during priming of naive CD8 cells.

In contrast to OX40-OX40L, the biology of 4-1BB appears very complex. Ligation of 4-1BB undoubtedly activates the proliferation, and the survival of CD8 T cells in vitro (11, 58–60). However, in vivo, agonist anti-4-1BB can lead to completely opposite effects. For example, anti-4-1BB can augment CTL generation and improve immunity against tumors and viruses (12–14), whereas in other cases it dramatically inhibits immune responses in several autoimmune models (30–34). One of the reasons might be the promiscuous expression of 4-1BB on many kinds of immune cells that have specific and perhaps opposing roles in various pathophysiologic circumstances. For example, it has been suggested that negative effects of anti-4-1BB could be due to promoting apoptosis of CD4 cells (34, 61), and that CD4 and CD8 cells respond differently to 4-1BB ligation (58). Alternatively, anti-4-1BB has been proposed to induce regulatory cells, either in the CD8 lineage (31, 62), or in the dendritic cell lineage (30).

In opposition to some of the experiments with anti-4-1BB, all studies of 4-1BBL−/− mice have either revealed no role for this ligand in initial CD8 priming, or suggested that 4-1BB/4-1BBL interactions play a positive, enhancing role, largely at a late stage of the CD8 T cell response when memory has formed or when memory is beginning to develop (23–27). Our studies represent the first to specifically address how the lack of 4-1BB on a CD8 cell affects its initial response and clearly show that without 4-1BB, CD8 cells are hyperresponsive to Ag in both primary and secondary responses, at least when provided via adenovirus. We do not believe this hyperresponsiveness is related to the Ag used in this study, or the vector, or the use of OT-I transgenic T cells, in that we have found similar hyperresponsiveness of 4-1BB−/− OT-I cells in mice immunized with OVA in CFA (data not shown), and enhanced CD8 priming to murine CMV in nontransgenic 4-1BB-deficient animals (L. Humphreys and M. Croft, unpublished observations). Moreover, these results agree well with our previous finding that 4-1BB−/− CD4 T cells from OT-II TCR transgenic mice also showed hyperresponsiveness to OVA protein in vivo (36). Thus, we conclude that 4-1BB, in physiological settings, can play a negative or limiting role in both CD4 and CD8 T cell responses, in addition to its apparent positive role, at least during the initial priming of effector T cells. It remains to be determined whether the loss of 4-1BB on CD8 cells affects the generation, maintenance, and responsiveness of long-term memory populations.

What then might explain an augmented T cell response when 4-1BB cannot be expressed? It is possible that in addition to the generation of positive signals through 4-1BB, there might be situations in which 4-1BB can transmit negative signals to a CD8 cell. This possibility would go along with the suggestion from autoimmune and graft-vs-host disease studies, in which anti-4-1BB is suppressive, that chronic 4-1BB signals may lead to apoptosis (activation-induced cell death) of pathogenic T cells (34, 61). This idea is feasible because we observed stable 4-1BB expression throughout the course of the primary adenovirus response. However, we failed to detect differences in apoptosis between wild-type and 4-1BB-deficient CD8 cells. Alternatively, a rate-limiting signal from 4-1BB might be due to the development of regulatory CD8 cells that are generated at the same time as effector CD8 cells. Thus, it is possible that some naive CD8 cells naturally take an alternate differentiation pathway enhanced by 4-1BB signals, and become regulatory. In the absence of 4-1BB, these cells would not then be generated, leading to enhanced effector CD8 responsiveness. Additional experiments will be needed to definitively conclude whether this mechanism is operative, but our phenotypic studies did not reveal any evidence of the presence/absence of potential regulatory populations. Lastly, another possibility stems from recent observations made with the TNFR family molecule, HVEM. HVEM has been thought to be costimulatory by interacting with LIGHT (63). However, HVEM−/− T cells were found hyperresponsive to stimuli, and HVEM−/− mice exhibited enhanced T cell-directed autoimmune hepatitis (64), somewhat similar to the phenotype we described previously with 4-1BB-deficient CD4 cells and in our study with 4-1BB−/− CD8 T cells. HVEM has now been shown to also bind the ITIM-containing B and T lymphocyte attenuator (BTLA) molecule that is expressed on both activated T cells and APC, and can transmit a negative signal, at least in part by recruiting the phosphatase Src homology protein-2 (65–67). Thus, HVEM interaction with LIGHT or BTLA resembles the interaction of B7-1-B7-2 with CD28 or CTLA4 (68), indicating that one molecule can bind partners driving either a stimulatory or inhibitory phenotype (69). It is an intriguing idea that the hyperresponsiveness of 4-1BB−/− T cells might be explained through 4-1BB possessing another ligand in addition to 4-1BBL that might provide a negative or modulatory...
influence in trans to other T cells or to APC, perhaps similar to BTLA. The fact that 4-1BB knockout and 4-1BBL knockout mice do not appear to phenocopy each other makes this possibility appealing.

In summary, we provide novel data to demonstrate that OX40 and 4-1BB physically operate in an opposing manner during initial priming of CD8 effector T cells. This study suggests the hypothesis that several TNFR family molecules, originally thought to play overlapping and potentially redundant roles, actually form an intricately balanced network. Some molecules might function only in a positive manner (e.g., OX40, CD27), whereas others might function positively or negatively (e.g., 4-1BB, HVEM), depending on either the stage of the T cell response or the availability of multiple opposing ligands, with their overall action being to fine-tune immune reactivity.

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Disclosures
The authors have no financial conflict of interest.

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