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OX40 Controls Functionally Different T Cell Subsets and Their Resistance to Depletion Therapy

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T cell depletion is a widely used approach in clinical transplantation. However, not all T cells are equally sensitive to depletion therapies and a significant fraction of T cells persists even after aggressive treatment. The functional attributes of such T cells and the mechanisms responsible for their resistance to depletion are poorly studied. In the present study, we showed that CD4+ T cells that are resistant to polyclonal anti-lymphocyte serum (ALS) mediated depletion exhibit phenotypic features of memory cells and uniformly express OX40 on the cell surface. Studies using the foxp3gfp knockin mice revealed that the remaining CD4+OX40+ cells consist of Foxp3+ Tregs and Foxp3− T effector/memory cells. The ALS-resistant CD4+OX40+ cells failed to mediate skin allograft rejection upon adoptive transferring into congenic Rag−/− mice, but removal of Foxp3+ Tregs from the OX40+ cells resulted in prompt skin allograft rejection. Importantly, OX40 is critical to survival of both Foxp3+ Tregs and T effector/memory cells. However, OX40 exhibits opposing effects on the functional status of Foxp3+ Tregs and T effector/memory cells, as stimulation of OX40 on T effector cells induced amplified cell proliferation but stimulation of OX40 on the Foxp3+ Tregs impaired their suppressor functions. Our study demonstrates that OX40 is a critical molecule in regulating survival and functions of depletion-resistant T cells; and these findings may have important clinical implications. The Journal of Immunology, 2007, 179: 5584–5591.

The size of T cell clones directed against fully MHC mismatched allografts is unusually large (1, 2). Thus, means to reduce the mass of alloreactive T cells are critical to the induction of transplant tolerance (3). In certain animal models, broad T cell depletion using cytolytic Abs sometimes creates tolerance (4), and in a selected cohort of patients, aggressive T cell depletion is often associated with delayed episodes of graft rejection, which can be managed with limited maintenance immunosuppression (5, 6). These findings lead to the belief that T cell depletion therapies may be protolerant in solid organ transplantation.

However, several surprising findings are recently reported concerning broad T cell depletion and tolerance induction in transplant models. First, peripheral T cells are inherently diverse and are not uniformly responsive to depletion therapies. For example, treatment of naive mice with cytolytic anti-CD4 and anti-CD8 (7), polyclonal anti-lymphocyte serum (ALS) (8), anti-thymoglobulin (ATG) (9) or CD3-immunotoxin fusion protein (4), though induces rapid and profound T cell depletion, fails to eliminate all the T cells, and a significant population of T cells persists in the periphery. Phenotypically, most of the remaining T cells express a surface phenotype that resembles effector memory T cells (9). Moreover, such depletion-resistant T cells can mediate prompt graft rejection response (7), suggesting that T cells that are remaining after depletion therapies are clearly alloreactive in transplant models. Similarly, in transplant patients who are treated with a depleting anti-CD52 (Campath-1H) or ATG as part of the induction therapy, T cells with effector memory properties are a predominant cell type remaining in treated recipients; and such remaining T cells exhibit potent alloimmunity (9). Second, depletion therapy using ALS can also spare a subset of Foxp3+ Tregs that are potentially immune suppressive in allograft rejection (8). In fact, sparing of the Tregs may be one of the mechanisms by which ALS treatment reverses autoimmune diabetes in overtly diabetic NOD mice (10, 11). Finally, in both humans and animal models, rejection mediated by depletion-resistant T cells is clearly different from that mediated by naive T cells, and the commonly used immunosuppressive drugs appear to function differently in regulating the effector functions of naive and depletion-resistant T cells (9). Thus, T cell depletion is not simply a reduction in cell numbers; depletion therapies appear to leave behind a complex repertoire of functionally competent T cells.

The key question that remains to be addressed is the precise mechanisms that render such T cells resistant to depletion therapies and the identity and functional attributes of those remaining T cells. In the present study, we sought to address this issue using recently generated new foxp3gfp knockin models and found that OX40 is a critical costimulatory molecule that defines a population of T effector cells and a population of Foxp3+ Tregs after broad T cell depletion. Importantly, OX40 plays an important role in survival of both T cell subsets but has strikingly opposing effects on their functions.

Materials and Methods

Animals

Male C57BL/6 (H-2b), DBA/2 (H-2b), and Rag−/− mice on the B6 background were purchased from The Jackson Laboratory. Generation of OX40−/− and OX40Ltg mice, all of which are on the C57BL/6 background, has already been described (12, 13). Foxp3-gfp knockin mice

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3 Abbreviations used in this paper: ALS, anti-lymphocyte serum; ATG, anti-thymoglobulin; KI, knockin; wt, wild type.

Results

Phenotypic features of CD4<sup>+</sup> T cells remaining after ALS treatment

Similar to our previous report (8), treatment of naive C57BL/6 mice with two doses of polyclonal ALS resulted in a rapid and profound reduction in T cells, and ~90% of the T cells in the spleen and the peripheral lymph nodes were depleted 5 days after ALS treatment (Fig. 1a). Phenotypic analysis of T cells that were depleted and those that were remaining in the CD4<sup>+</sup> compartment at a time of maximal depletion showed that ALS preferentially depleted the CD4<sup>+</sup>CD62L<sup>−</sup> naive T cells in the spleen while all the remaining CD4<sup>+</sup> T cells were CD4<sup>+</sup>CD62L<sup>+</sup> (Fig. 1b), confirming that the remaining CD4<sup>+</sup> T cells express features of memory cells. Similar finding was observed in the peripheral lymph nodes of ALS-treated mice (data not shown). This is consistent with other reports that memory T cells are a predominant cell type remaining after aggressive depletion (9). Interestingly, the remaining CD4<sup>+</sup> T cells were found to constitutively express OX40 on the cell surface (Fig. 1c). As OX40 is critical to the generation/survival of CD4<sup>+</sup> memory T cells in other models (17, 18), expression of OX40 by such depletion-resistant T cells suggests that OX40 may play an important role in survival or function of those CD4<sup>+</sup> T cells.

Role of CD4<sup>+</sup>OX40<sup>+</sup> T cells remaining after ALS treatment in mediating skin allograft rejection

To determine the functional attributes of CD4<sup>+</sup>OX40<sup>+</sup> T cells remaining after ALS treatment in transplant models and possible means to target these cells, we FACS sorted CD4<sup>+</sup>OX40<sup>+</sup> T cells from ALS-treated B6 mice and adoptively transferred them into syngeneic Rag<sup>−/−</sup> mice. The host mice were then grafted with fully MHC mismatched DBA/2 skin allografts, and graft survival was determined and compared with mice transferred with freshly prepared CD4<sup>+</sup>OX40<sup>−</sup> T cells. As shown in Fig. 2, Rag<sup>−/−</sup> mice transferred with CD4<sup>+</sup> T cells or CD4<sup>+</sup>OX40<sup>−</sup> T cells readily rejected the DBA/2 skin allografts with a mean survival time of 22 days (n = 4 to 7). To our surprise, all the Rag<sup>−/−</sup> mice transferred with CD4<sup>+</sup>OX40<sup>+</sup> T cells accepted the skin allografts long-term (>100 days, n = 9) without any signs of rejection. The lack of rejection is not due to the cell numbers transferred, as transferring as high as 1 million CD4<sup>+</sup>OX40<sup>+</sup> T cells also failed to trigger a rejection response (data not shown).

OX40 defines a subset of CD4<sup>+</sup> T effector cells and Foxp3<sup>+</sup> Tregs

To probe the paradox of CD4<sup>+</sup>OX40<sup>+</sup> T cells that express a memory phenotype but fail to mediate rejection, we first examined whether CD4<sup>+</sup>OX40<sup>+</sup> T cells would contain a subset of Foxp3<sup>+</sup> T cells.
Each point represents 3 to 5 mice. Spleen were determined and plotted against the untreated control mice.

CD44 and OX40 on the remaining CD4 T cells were prepared from B6 mice 5 days after ALS treatment, and expression of these genes were shown are representative of four independent experiments.

ALS were sacrificed at different time points, CD4 T cells from ALS-treated C57BL/6 mice were prepared and labeled with CyChrome-anti-CD4 and PE-anti-CD62L, the surface phenotype of ALS-resistant T cells before and after ALS treatment. Spleen cells from ALS-treated C57BL/6 mice were prepared and labeled with CyChrome-anti-CD4 and PE-anti-CD62L, the surface phenotype of ALS-resistant T cells was analyzed by FACS and compared with T cells from untreated control mice. All analyses were performed by gating onto the CD4 fraction. Data shown are representative of four independent experiments. Spleen cells were prepared from B6 mice 5 days after ALS treatment, and expression of CD44 and OX40 on the remaining CD4 T cells was analyzed and shown. Spleen cells from untreated control mice were included as a control. Data shown are representative of three independent experiments.

Tregs that can suppress the T effector cell functions. For this purpose, we took advantage of the newly generated foxp3-gfp.KI mice in which the endogenous Foxp3 is genetically linked to a reporter protein EGFP (14) and treated the foxp3-gfp.KI mice with the same ALS protocol, we then analyzed the presence of GFP-

FIGURE 1. Surface phenotype of CD4 T cells resistant to ALS-mediated depletion. a, Time dependent changes in CD4 T cells in the host spleen after ALS treatment. Naive C57BL/6 mice treated with two doses of ALS were sacrificed at different time points, CD4 T cells in the host spleen were determined and plotted against the untreated control mice. Each point represents 3 to 5 mice. b, Surface phenotype of CD4 T cells before and after ALS treatment. Spleen cells from ALS-treated C57BL/6 mice were prepared and labeled with CyChrome-anti-CD4 and PE-anti-CD44 or PE-anti-CD62L, the surface phenotype of ALS-resistant T cells was analyzed by FACS and compared with T cells from untreated control mice. All analyses were performed by gating onto the CD4 + fraction. Data shown are representative of four independent experiments. c, Spleen cells were prepared from B6 mice 5 days after ALS treatment, and expression of CD44 and OX40 on the remaining CD4 T cells was analyzed and shown. Spleen cells from untreated control mice were included as a control. Data shown are representative of three independent experiments.

FIGURE 2. Role of ALS-resistant CD4+OX40+ T cells in mediating skin allograft rejection. B6 mice were treated with two doses of ALS. Five days later, CD4+OX40+ T cells were FACS sorted and adoptively transferred into syngeneic B6.Rag−/− mice via the tail vein (2 × 10⁶ cells/mouse). The host mice were grafted with DBA/2 skin allografts on the same day of cell transfer, and skin allograft survival was determined and compared with mice transferred with CD4+ T cells or CD4+OX40− T cells. The skin allograft survival was presented in a Kaplan-Meier plot.
FIGURE 3. Phenotypes and functions of CD4⁺ T cells resistant to ALS-mediated depletion. a, Both Foxp3⁺ Tregs and Foxp3⁻ T effector cells are resistant to ALS. Naive Foxp3gfp.KI mice were treated with two doses of ALS. Spleen cells from the treated mice were prepared 5 days later and labeled with PE-anti-CD3 and CyChrome-anti-CD4. Cells expressing both CD3 and CD4 were selectively gated and further analyzed for the presence of GFP(Foxp3)⁺ Tregs and GFP(Foxp3)⁻ T effector cells by FACS. Spleen cells from untreated Foxp3gfp.KI mice were included as a control. The dot plot shown is representative of four independent experiments. The absolute number of CD4⁺GFP⁻ T cells and CD4⁺GFP⁺ T cells was calculated based on the total number of cells recovered from each spleen and the percentage of CD4⁺ fractions in the recovered cells. b, Expression of Treg-related gene transcripts by ALS-resistant CD4⁺OX40⁺ T cells. CD4⁺OX40⁺ T cells were FACS sorted from the ALS-treated B6 mice 5 days after ALS treatment, CD4⁺CD25⁻ Tregs and CD4⁺CD25⁺ T effector cells were sorted from naive B6 mice and used as controls. Expression of Foxp3, CTLA-4, GITR, CD25, and CD103 gene transcripts in the sorted T cells was quantitated simultaneously by real-time PCR. The histogram shown is a representative plot of three experiments. c, T cell suppression assay in vitro. Wild-type foxp3gfp.KI mice were treated with ALS, spleen cells were prepared from the treated mice 5 days later, and CD4⁺GFP(Foxp3)⁺ Tregs and CD4⁺GFP(Foxp3)⁻ T effector cells were FACS sorted. The sorted cells (1 × 10⁵) were mixed at 1:1 ratio and stimulated with anti-CD3 (2 ug/ml) plus equal number of APCs for 3 days. Cell proliferation was determined by [³H]TdR uptake. Data shown are mean cpm ± SD of triplicate assays. d, Coexpression of CD25 and Foxp3 by Tregs. CD4⁺ T cells were selectively gated and expression of CD25 and Foxp3 was analyzed by FACS and shown. e, Skin allograft survival in B6.Rag⁻/⁻ mice transferred with ALS-resistant CD4⁺OX40⁺ T cells with or without depletion of CD25⁺ Tregs. Naive B6 mice were injected with a depleting anti-CD25 mAb (PC61, 0.25 mg i.p. on days −2 and 0) to deplete CD25⁺ Tregs, followed by ALS treatment. Five days later, CD4⁺OX40⁺ T cells were FACS sorted from the treated mice and adoptively transferred into syngeneic Rag⁻/⁻ mice. The host mice were grafted with DBA/2 skin allografts on the day of cell transfer and graft survival was determined and shown. *, p < 0.05.
FIGURE 4. Effect of ALS-mediated CD4+ T cell depletion in wt and OX40−/− mice. Wt foxp3-gfp.KI mice and OX40−/− foxp3gfp.KI mice were treated with the same ALS protocol. Spleen and LN cells were prepared five days later and briefly stained with PE-anti-CD3 and CyChrome-anti-CD4. CD4+ T cells were then selectively gated and expression of GFP(Foxp3) within the CD4+ fraction was analyzed and shown. Untreated wt and OX40−/− foxp3-gfp.KI mice were used as controls. The absolute number of CD4+GFP+ T cells and CD4+GFP− T cells was calculated based on the total number of cells recovered from each mouse and the percentage of CD4+ fractions in the recovered cells. Data shown are representative of three independent experiments. *, p < 0.05.

FIGURE 5. Effect of ALS-mediated CD4+ T cell depletion in wt and OX40Ltg mice. Wt foxp3-gfp.KI mice and OX40Ltg-foxp3gfp.KI mice were treated with the same ALS protocol. Spleen and LN cells were prepared five days later and briefly stained with PE-anti-CD3 and CyChrome-anti-CD4, CD4+ T cells were then selectively gated and expression of GFP(Foxp3) within the CD4+ fraction was analyzed and shown. Untreated wt and OX40Ltg-foxp3-gfp.KI mice were used as controls. The absolute number of CD4+GFP+ T cells and CD4+GFP− T cells was calculated based on the total number of cells recovered from each mouse and the percentage of CD4+ fractions in the recovered cells. Data shown are representative of three independent experiments. *, p < 0.05.
OX40 is critical to survival of both CD4$^+$ T effector cells and Foxp3$^+$ Tregs

Clearly, CD4$^+$ T effector cells and Foxp3$^+$ Tregs that are resistant to ALS depletion constitutively express OX40. Although it is known that OX40 delivers a potent costimulatory signal to T effector cells (19), little is known as to whether OX40 would differentially regulate the survival of Foxp3$^+$ Tregs and Foxp3$^-$ T effector cells in response to ALS-mediated depletion. To address this issue, wt foxp3-gfp.KI and OX40$^{-/-}$ foxp3-gfp.KI mice were treated with the same ALS protocol, and survival of Foxp3$^+$ Tregs and Foxp3$^-$ T effector cells in the remaining CD4$^+$ T cells was analyzed 5 days later and compared simultaneously. As shown in Fig. 4, wt control mice and OX40$^{-/-}$ mice have comparable levels of CD4$^+$ T cells. However, as compared with wt mice, treatment with ALS resulted in a drastic reduction of CD4$^+$ T cells in OX40-deficient mice; and very few CD4$^+$ T cells were identified in ALS-treated OX40-deficient mice. Interestingly, both CD4$^+$Foxp3$^+$ Tregs and CD4$^+$Foxp3$^-$ T effector cells were equally depleted by ALS in OX40-deficient mice (Fig. 4).

To further determine the role of OX40/OX40L pathway in the survival of CD4$^+$ T cells after ALS treatment, we treated the OX40Ltg-foxp3gfp.KI mice with the identical ALS protocol and examined the remaining CD4$^+$ T cells with that of wt control mice. In striking contrast to the wt controls, T cells in OX40Ltg foxp3-gfp.KI mice were highly resistant to ALS-mediated depletion, and very few CD4$^+$ T cells in the OX40Ltg mice were depleted by ALS (Fig. 5). As compared with the wt controls, CD4$^+$ T cells in OX40Ltg mice showed noticeable expansion. Interestingly, both CD4$^+$Foxp3$^+$ Tregs and CD4$^+$Foxp3$^-$ T effector cells in the OX40Ltg mice are resistant to ALS-mediated depletion. These data suggest that OX40/OX40L pathway is critical to survival of both Foxp3$^+$ Tregs and Foxp3$^-$ T effector cells in vivo.

Real-Time RT-PCR analysis showed that OX40Ltg T cells constitutively express high levels of Survivin and Bcl-XL when compared with wt T cells or OX40-deficient T cells (Fig. 6), suggesting that the survival advantage of OX40Ltg T cells may be mediated in part by over-expression of cell survival molecules.

OX40 has opposing effects on the functions of CD4$^+$ Foxp3$^-$ T effector cells and Foxp3$^+$ Tregs

OX40 is expressed by both Foxp3$^-$ T effector cells and Foxp3$^+$ Tregs remaining after ALS treatment. To determine the role of
OX40 in regulating the functional attributes of T effector cells and Foxp3+ Tregs. wt foxp3-gfp.KI mice were treated with ALS, CD4+Foxp3− T effector cells and CD4+Foxp3+ Tregs were sorted 5 days later, and the effect of OX40 stimulation on their functions was analyzed in vitro using an agonist anti-OX40 mAb (clone OX86). As shown in Fig. 7a, stimulation of OX40 on the T effector cells resulted in an amplified proliferative response, consistent with a costimulatory role of OX40 to the T effector cells. However, in multiple suppression assays using OX40KO T effector cells as responding cells, selective stimulation of OX40 on the Foxp3+ Tregs consistently impaired their suppressor functions in suppressing T effector cell proliferation (Fig. 7b), which is consistent with recent reports using Foxp3+ Tregs from unmanipulated mice (20, 21). Thus, OX40 appears to have opposing effects on Foxp3− T effector cells and Foxp3+ Tregs.

Discussion
Understanding precisely the functional attributes of T cells remaining after depletion therapies and the molecular pathways involved in their resistance is an important and clinically relevant issue in transplantation. In the present study, we attempted to address this issue using recently created new models and uncovered several novel and interesting findings. First, CD4+ T cells remaining after ALS treatment, though phenotypically resembling effector/memory cells, are in fact heterogeneous and consist of two functionally different T cell subsets (i.e., T effector cells and Foxp3+ Tregs). Second, Foxp3+ Tregs in the remaining CD4+ T cells are fully functional because they can effectively suppress T effector cell-mediated rejection in vivo in an adoptive transfer model. Third, OX40 is constitutively expressed by both T cell subsets and plays a critical role in their survival and resistance to depletion therapy. Finally, OX40 delivers a potent costimulatory signal to the T effectors but appears to be a negative regulator of Foxp3+ Tregs, which is a striking feature of OX40 in this setting.

There are several reports in the literature showing that T cells in the periphery are not uniformlyresponsive to depletion therapies, and a significant fraction of T cells persists in the periphery regardless of depleting reagents used and the modes of treatment (4–6, 8, 9, 22). However, the identity of T cells remaining after depletion therapies and their functional attributes are poorly characterized and tend to differ in different studies (8, 9). In the present study, we demonstrated that CD4+ T cells that are resistant to ALS treatment are phenotypically memory-like cells as the T cells remaining after ALS treatment are uniformly CD44highCD62L−, which is consistent with several previous reports (9, 22). But functionally, they consist of two distinct T cell subsets (i.e., Foxp3+ Tregs and Foxp3− T effectors), such a parity is strikingly clear in foxp3-gfp.KI mice in which Foxp3+ Tregs are genetically marked by the expression of GFP protein (14). At a population level, Foxp3+ Tregs only account for a fraction of OX40+ T cells remaining after ALS treatment (∼30%), but they appear to be functionally predominant as adoptive transfer of OX40+ T cells into Rag−/− mice failed to trigger the skin allograft rejection response. However, the Foxp3− T effector cells in the OX40− fraction are fully competent as removal Foxp3+ Tregs from the OX40− T cells precipitates rapid skin allograft rejection. It is important to emphasize that the Foxp3+ Tregs are not locked into a dominant status at all times. For example, stimulating OX40 using an agonist mAb can trigger potent effector function in the secondary hosts upon adoptive transfer. Also, when CD4+OX40− T cells are allowed to undergo homeostatic proliferation first in Rag−/− hosts for 4 to 6 wk, followed by grafting the MHC mismatched skin allografts, graft rejection readily ensures (our unpublished observation). Thus, the outcome of the allograft response depends on a delicate balance between such functionally different T cell subsets, and this balance is metastable and can be regulated by other factors.

One of the key findings of our study is that OX40 is a critical pathway to the survival of CD4+ T cells remaining after ALS treatment. This notion is based on the observation that treatment of OX40−/− foxp3-gfp.KI mice, in contrast to the wt controls, resulted in a drastic reduction of the remaining CD4+ T cells. In fact, very few CD4+ T cells remained in the OX40−/− mice after ALS treatment. In stark contrast, ALS largely failed to deplete CD4+ T cells in OX40Ltg mice. Thus, OX40 appears to be a key survival molecule for the CD4+ T cells in resistance to ALS-mediated depletion. This finding also suggests that in ALS-treated mice, survival of both T effector cells and Foxp3+ Tregs is reliant on OX40 expression despite their functional differences. The precise mechanisms that OX40 confers resistance of CD4+ T cells to depletion therapy are not clear, but may be related to the survival effect of OX40 costimulation. OX40 is originally identified as a T cell activation marker, as OX40 is highly expressed on activated, but not resting T cells (23). It is now known that OX40 is a potent T cell costimulatory molecule and regulates multiple aspects of the T cell response (18, 19). OX40 is important in survival of activated T cells, most likely by sustaining the expression of Bcl-Xl and Survivin (24, 25), all of which are potent anti-apoptotic molecules. OX40 is also critical to the generation and survival of memory CD4+ T cells (26, 27). These features are clearly germane to the findings reported in our study. However, it remains unclear as to what triggers the initial expression of OX40 in seemingly naive mice. T cells can undergo homeostatic proliferation in immunodeficient mice or upon broad T cell depletion, which may render the remaining T cells to up-regulate certain cell surface molecules following homeostatic expansion (7, 22). In our study, however, homeostatic proliferation is unlikely a major contributing issue as all analyses were performed at a time point when maximal T cell reduction is induced by treatment with ALS. In this model, homeostatic proliferation of residual T cells usually takes much longer to occur (28, 29). The question as to whether OX40 is expressed by all types of memory T cells or only a subset of memory cells remains to be defined. More studies are clearly warranted to further unravel these issues.

Our study has several important clinical implications. Therapeutic strategies to target T cells after broad T cell depletion in transplantation should take into account the functional heterogeneity of such T cells. It would be highly desirable to selectively block T effector cells without compromising the Foxp3+ Tregs. In the clinical setting, Pearl et al. reported that memory CD4+ T cells remaining after depletion therapies are highly resistant to commonly used immunosuppressive drugs in vitro, but proliferation and effector functions of such CD4+ T cells are particularly sensitive to calcineurin inhibitors (9). This led to the proposition that treatment with calcineurin inhibitors may be ideal to contain post depletion T cells. However, calcineurin inhibitors are also recognized as highly detrimental to the Foxp3+ Tregs (30). Thus, in the setting of broad T cell depletion, treatment with calcineurin inhibitors, while inhibiting the memory-like T effector cells may also interfere with the suppressor functions of Foxp3+ Tregs. Our data suggest that OX40 exhibits opposing effects on T effector cells and Foxp3+ Tregs, and targeting this pathway may be therapeutically important. However, as OX40 is critical to the survival of both subsets, prolonged OX40 blockade may also compromise the survival of Foxp3+ Tregs. Whether the timing and the duration of treatment will favor differential survival of T effector cells vs Tregs requires further investigation.
In conclusion, our findings reinforce the notion that aggressive T cell depletion leaves behind a diverse T cell subsets with strikingly different functional attributes. OX40 is a critical molecule that defines a population of T effector cells and Foxp3+ Tregs after broad T cell depletion. Importantly, OX40 plays an important role in survival of both T cell subsets but has strikingly opposing effects on their functions. These findings may have important clinical implications aimed at modulating both effector T cells and regulatory T cells in tolerance induction.

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Disclosures

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