OX40 Signals during Priming on Dendritic Cells Inhibit CD4 T Cell Proliferation: IL-4 Switches off OX40 Signals Enabling Rapid Proliferation of Th2 Effectors

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OX40 Signals during Priming on Dendritic Cells Inhibit CD4 T Cell Proliferation: IL-4 Switches off OX40 Signals Enabling Rapid Proliferation of Th2 Effectors

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In this study we examined the role and regulation of OX40 signals during CD4 T cell priming on dendritic cells (DCs). Contrary to expectation, OX40-deficient cells proliferated more rapidly than their normal counterparts, particularly when stimulated with peptide in the absence of added cytokines. This proliferative advantage was not apparent for Th2-differentiated cells. When the reasons for this were investigated, we found that the cytokine IL-4 specifically down-regulated expression of OX40 ligand on T, B, and DCs, but not on the CD4⁺CD3⁺ cells linked with selection of Th2 cells into the memory compartment. OX40 ligand expression was also down-regulated on rapidly proliferating Th1 effectors. These data are compatible with OX40 signals acting during priming as a check on naive T cell proliferation while T cells integrate additional DC signals. This would serve to limit inappropriate T cell responses. In contrast, OX40 signals from CD4⁺CD3⁺ cells located in the outer T zone select proliferating Th2 effectors into the memory T cell pool. The Journal of Immunology, 2005, 174: 1433–1437.

The development of protective CD4 immune responses depends on a combination of Ag-specific, costimulatory, and cytokine signals at the time T cells are primed on dendritic cells (DCs). This determines effector differentiation and subsequently entry into the memory T cell compartment. OX40 signals have been implicated both during DC priming and in memory maintenance.

The evidence for OX40 involvement in CD4 T cell memory comes from studies using mice deficient in OX40 signals: CD4 recall responses were significantly impaired (1–3). This was further substantiated by demonstrating that OX40 signals up-regulated Bcl-xL and Bcl-2, so providing survival signals to CD4 T cells (4). Recently, we reported that the OX40 survival signals can be provided to Th2 primed CD4 T cells by a CD4⁺CD3⁺CD11c⁺ accessory cell that constitutively expresses OX40 ligand (5). These cells are located at the border of T cell areas and in B follicles, so do not normally interact with naive T cells during priming on DCs.

The role of OX40 signals during T cell priming on DCs is more controversial. The ligand is expressed on CD40-activated DCs (6, 7) but also activated T cells (reviewed in Ref. 8). There is evidence from in vivo studies that T cells receiving OX40 signals during priming on DCs favor Th2 differentiation. This is exemplified by the murine strain-dependent immune response to Leishmaniasis. Whereas C57BL/6 mice develop protective Th1-mediated immune responses, the early production of IL-4 in BALB/c mice by a distinct subset of CD4 T cells (9, 10), skews T cell responses away from protective Th1 mediated immunity (11). Blockade of OX40 signals with Abs allows BALB/c mice to develop protective Th1 immunity (3), whereas constitutive expression of OX40 ligand on T cells renders C57BL/6 mice (normally resistant to disease) susceptible, because of excess production of Th2 cytokines (12). The Th2 skewing observed in vivo is also observed in vitro (13–15).

There are also many studies linking OX40 signals with autoimmunity and by inference Th1 responses. Injection of anti-OX40 ligand Abs abrogated the development of inflammatory bowel disease (16) and experimental allergic encephalomyelitis (17). Although these findings could have been due to depletion of T cells expressing OX40 ligand, there are now studies showing OX40 signals are essential for the development of diabetes in NOD mice (18). Paradoxically, in the last model, OX40-deficient T cells proliferated more rapidly than normal T cells and invaded pancreatic tissue with faster kinetics, but failed to lead to destructive lesions in the islets.

Published data concerning the role of OX40 in Th1 and Th2 responses are thus apparently contradictory. In this paper, using transgenic T cells deficient and sufficient in OX40 signals, we have asked three distinct questions to help resolve this paradox. 1) What is the effect of OX40 signals during T cell priming on DCs in directing Th1 vs Th2 differentiation? 2) What is the effect of OX40 signals on T cell proliferation? 3) Do cytokines regulate OX40 signals from DCs and CD4⁺CD3⁺ cells? We found that the proportion of T cells that could be induced to produce Th2 (IL-4) or Th1 (IFN-γ) cytokines did not depend on OX40 signals. Furthermore, as has been reported by others (19), the initial proliferation of T cells when primed under immunizing conditions in vivo did not depend on intact OX40 signals. However, when equal numbers of OX40-deficient and normal T cells were stimulated in vitro, under Th1 and particularly Th0 conditions, OX40-deficient cells underwent a greater number of divisions than their normal counterparts. In contrast, under Th2 conditions, both populations of T cells proliferated to the same extent. When the reasons for this were investigated, it was clear that T cells or DCs stimulated in the presence of IL-4 abrogated their expression of OX40 ligand, and this was associated with the capacity of normal T cells to proliferate as rapidly as their OX40-deficient counterparts. In contrast to DCs, the expression of OX40 ligand on CD4⁺CD3⁺ cells was not

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3 Abbreviation used in this paper: DC, dendritic cell.
affected. These results are discussed in the light of the regulation of T cell help for Th1 and Th2 responses.

Materials and Methods

Mice

Normal and RAG1-deficient (RAG1<sup>−/−</sup>) C57BL/6 mice were bred and maintained in our animal facility. OX40-deficient mice (1) were crossed with the OVA-specific TCR transgenic (OTII) mice (20) and were homozygous for the expression of CD45.2. Homozygous OTII mice were also crossed with CD45.1 congenic C57BL/6 mice to obtain allotype marked OVA-specific transgenic T cells that could be distinguished in cultures from OX40-deficient CD45.2 OTII cells. All mice were used between 6 and 16 wk of age and were sex and age matched as far as possible in individual experiments. All mice were housed and maintained in the University of Birmingham, Biomedical Services Unit (Birmingham, U.K.).

T cell stimulation and surface staining

Splenocytes were collected from OX40-sufficient (CD45.1) or -deficient (CD45.2) OTII transgenic mice. For Th0-, Th1-, and Th2-polarized cell cultures, OX40<sup>+</sup> and OX40<sup>−</sup> cells were cultured alone or in the mixture. For mixed culture, OX40<sup>+</sup> and OX40<sup>−</sup> were mixed 1:1 (total 1 × 10<sup>6</sup> cells/ml/well) and the final ratio of CD4 T cells was determined by flow cytometry. Mixtures were incubated in RPMI 1640 medium containing l-glutamine, and 10% FCS with 0.01–10 μM OVA<sub>23–39</sub> peptide at 37°C with 5% CO<sub>2</sub> (Th0 conditions). For Th1 conditions, 10 ng/ml IL-12 (Peprotech) and 10 μg/ml anti-IL-4 mAb were added to cultures, and for Th2 conditions, 10 ng/ml IL-4 (Peprotech) and 10 μg/ml anti-IL-12 mAb were added. Cultured cells were stained to detect the surface expression of TNFR and ligands.

Abs for staining

Anti-CD4 PE, anti-CD8 APC, anti-CD11c PE, anti-CD3 APC, anti-CD45.1 FITC, anti-CD45.1 PE, anti-B220 FITC, anti-IL-4 APC, and anti-IFN-γ APC mAbs and biotinylated mAb against CD3, OX40, OX40 ligand, CD30, CD30 ligand, 4-BB, 4-BB ligand, CD11c, and CD70 were purchased from BD Pharmingen. Anti-TRANCE biotinylated mAb was obtained from R&D Systems. Anti-CD8α FITC mAb was obtained from Caltag Laboratories. As the second step staining reagents, streptavidin CyChrome was purchased from BD Pharmingen.

CFSE labeling

Splenocytes collected from OX40-sufficient (CD45.1) or -deficient (CD45.2) OTII transgenic mice were labeled with intracellular fluorescent dye CFSE (Molecular Probes). Briefly, cells were washed twice in PBS containing 0.1% BSA and resuspended at 10<sup>7</sup> cells/ml in PBS containing 0.1% BSA with 10 μM CFSE for 10 min at 37°C. Cells were washed twice with RPMI medium and then in vitro cultured under Th0, Th1, and Th2 conditions with 0.01–10 μM OVA<sub>23–39</sub> peptide. After 3 or 4 days, cell divisions were analyzed by flow cytometry using a FACScan (BD Biosciences).

For in vivo transfer experiments, a final mixture of 1:1 OX40-sufficient:OX40-deficient cells (total 1 × 10<sup>7</sup> cells/mouse) was labeled with CFSE and transferred into RAG1<sup>−/−</sup> mice. The mice were immunized the next day with 200 μg alum-precipitated OVA. Some mice were not immunized as controls. On day 3, spleens were taken and the dilution of the CFSE dye in dividing populations of T cells was checked by flow cytometry.

Preparation and activation of CD4<sup>+</sup>CD3<sup>+</sup> cells and DCs

Cell suspensions for isolation of CD4<sup>+</sup>CD3<sup>+</sup> cells and DCs were made from the spleens of RAG1<sup>−/−</sup> mice as follows. Spleens were cut into small fragments and then cultured with collagenase D (1 mg/ml; Boehringer Mannheim) for 45 min at 37°C. Digested fragments were crushed between gauze. After depletion of RBC with Gey’s solution, the cell suspensions were pooled and resuspended in MACS buffer containing 10% mouse serum as per manufacturer’s instructions. CD11c<sup>+</sup> cells were enriched by incubating the cell suspension with MACS anti-mouse CD11c microbeads (Miltenyi Biotec) at 100 μl/10<sup>6</sup> cells for 15 min at 4°C. CD11c<sup>+</sup> bead-attached single cells were positively selected using autoMACS (Miltenyi Biotec) with possel mode. CD4<sup>+</sup> cells from CD11c<sup>+</sup> cells were enriched as above and positively selected with possel-D mode. The resulting populations and OX40 ligand expression on CD4<sup>+</sup> cells were analyzed on a BD FACSCalibur (BD Biosciences), and 10 μg/ml anti-CD40. After 2 days, CD8<sup>+</sup>CD11c<sup>+</sup> and CD8<sup>+</sup>CD11c<sup>+</sup> DCs from the CD11c-enriched population, and CD4<sup>+</sup>CD3<sup>+</sup> cells from the CD4-enriched population were stained with appropriate Abs and analyzed by flow cytometry. For B cell analysis, splenocytes were prepared from normal mice and cultured alone or with an activation agent as described above.

Results

Th1 and Th2 differentiation in OX40-deficient and -sufficient cells

To test directly the effects of OX40 signals during T cell differentiation, we mixed normal and OX40-deficient transgenic T cells in a 1:1 ratio and compared their cytokine profiles and proliferation at different times after stimulation. Mixtures of transgenic T cells were stimulated with peptide and either 1) IL-4 and anti-IL-12 mAb (Th2 conditions) or 2) IL-12 and anti-IL-4 mAb (Th1 conditions). A similar proportion of Th1-differentiated OX40-deficient and normal T cells expressed IFN-γ (Fig. 1a). Although in paired experiments, a statistically significant higher proportion of normal cells expressed IL-4, these differences were small, and it is quite clear that OX40 is not essential for IL-4 production provided IL-4 is provided exogenously in the starting cultures (Fig. 1b).

The striking finding is the proportions of normal and OX40-deficient cells in the cultures. After 6 days of culture under Th1 conditions of differentiation, OX40-deficient cells exceed their normal counterparts by ∼3:1, and the overall numbers of OX40-deficient cells expressing IFN-γ were significantly greater (Figs. 1c and 2a). Although Th0-differentiated cells (peptide alone) did not secrete either IFN-γ or IL-4 (data not shown), OX40-deficient cells outnumbered normal cells by a more substantial margin, ∼5:1 (Fig. 2a). Similar results were obtained if OX40-deficient and -sufficient cells were cultured separately (data not shown). In contrast, Th2-differentiated cells did not show such a bias (OX40-deficient:OX40-sufficient ratio, 1:1:1). These data suggest that OX40 signals are not essential for Th1 or Th2 differentiation. The unexpected finding was the increased numbers of Th1- and particularly Th0-differentiated OX40-deficient cells.
compared with control Ab staining.

In immunized with alum-precipitated OVA 1 day later. Transferred OTII T cells were gated with CD4-positive and CD3-positive cells (red circle). In proliferation of T cells was indeed OX40 independent (Fig. 2).

creased proliferation of OX40-deficient cells or due to decreased survival of OX40-sufficient cells, we repeated the experiment using T cells labeled with the vital membrane dye, CFSE. OX40-deficient and -sufficient OTII transgenic T cells were mixed in 1:1 ratio; labeled with the membrane dye, CFSE; and stimulated with 1 μM OVA peptide. Shown is the division history for OX40-deficient cells (red) vs normal (blue) after 3 days of activation. Numbers show the average ratio (n = 10) of OX40-deficient: normal cells for Th0-, Th1-, and Th2-differentiated cells. b. Day 3 CFSE labeling of normal (blue line) and OX40-deficient (red line) T cells. A 1:1 mixture of OX40-deficient and allotype marked normal cells were transferred into RAG1–/– mice, which were then immunized with alum-precipitated OVA 1 day later. Transferred OTII T cells were gated with CD4-positive and CD3-positive cells (red circle). In the absence of immunization, CFSE labeling of the transferred OTII cells remained uniformly high, indicating that they did not divide (black line). c. Expression of OX40 and OX40 ligand (OX40L) on day 3 on Th0-, Th1-, and Th2-differentiated OTII cells. Numbers show the percent positive cells compared with control Ab staining. d. Expression of OX40 ligand vs division history assessed by CFSE staining.

OX40-deficient CD4 T cells undergo more divisions when stimulated with Ag under Th1 and Th0 but not Th2 conditions

The above data do not fit with the conventional wisdom that OX40 signals are costimulatory to T cells. To test directly whether the results that we had obtained in vitro were mirrored in vivo, we transferred mixtures of CFSE-labeled OX40-deficient and -sufficient transgenic CD4 T cells into RAG1–/– recipient mice. After immunization with the Th2 Ag, alum-precipitated OVA, the initial proliferation of T cells was indeed OX40 independent (Fig. 2b), mimicking what we had observed under Th2 conditions of immunization in vitro. Proliferation of T cells did not occur in the absence of immunization.

Previous studies indicated that Ag-specific OX40-deficient T cells that encountered Ag expressed in self tissues proliferated more rapidly than their normal counterparts in vivo (18). This is the in vivo equivalent of our Th0 conditions in vitro. To clarify whether the increased numbers of OX40-deficient cells differentiated under Th0 conditions that we had observed was due to increased proliferation of OX40-deficient cells or due to decreased survival of OX40-sufficient cells, we repeated the experiment using T cells labeled with the vital membrane dye, CFSE. OX40-deficient and -sufficient T cells mixed to give a starting ratio of 1:1 were stimulated over a 1000-fold range of peptide concentrations (from 0.1 to 100 μM), over which the pattern of proliferation of OX40-deficient cells relative to OX40-deficient cells was similar (data not shown). Fig. 2a shows CFSE labeling at 1 μM peptide.

The number of cell divisions undergone by Th2 primed OX40-deficient and -sufficient T cells was similar, although on average a slightly higher number of OX40-deficient cells were present in cultures after 6 days (1.1:1; n = 10). In contrast, OX40-deficient Th0 and OX40-deficient Th1 cells went through a greater number of divisions than their OX40-sufficient counterparts and this was reflected by the average ratio of OX40-deficient:OX40-sufficient cells on day 6 cultures: 4.7:1 for Th0 and 3.1:1 for Th1 cells (n = 10). The impaired proliferation of OX40-sufficient cells was specifically due to signals through OX40 and not due to “reverse signaling” through the ligand, because the coculture of OX40-sufficient and -deficient cells ensures that OX40-deficient cells can receive signals through OX40 ligand from adjacent OX40 expressing cells. This finding shows that OX40 does not act as a conventional costimulatory molecule (e.g., CD28), for it neither alters the threshold for T cell activation, nor increases the extent of proliferation in dividing cells. It is consistent with the paradoxical in vivo observation, that OX40-deficient T cells transferred into recipient mice proliferate more rapidly (18).

OX40 ligand is expressed on Th0 and Th1 cells, but is down-regulated on differentiating Th2 cells

OX40 ligand is up-regulated (within 24 h) on CD40-activated DCs (6, 7), and OX40 is expressed on T cells within 48 h of activation (14) and therefore can exert their effects on proliferation observed by day 3. These data suggested that OX40 signals delivered at the time T cells engage DCs inhibit proliferation. Th0-, Th1-, and Th2-differentiated T cells all expressed OX40 by 48 h (data not shown) but by day 3 Th2 cells were the most strongly positive. Although difficult to detect in vivo, T cell expression of OX40 ligand has been described (8). In vitro, OX40 ligand expression was readily detected 48 h following T cell activation, particularly on Th0 but also on Th1-differentiated T cells. In contrast, Th2 cells lacked OX40 ligand expression by day 3 (Fig. 2c). A kinetic study of OX40 ligand and receptor expression on CD4 OTII cells showed that on day 1 expression of OX40 ligand was comparable on Th1, Th0, and Th2 cells, but by day 3, expression was lost from Th2 cells (data not shown).

OX40 ligand is down-regulated on proliferating Th1 but not Th0 cells

Using CFSE-labeled cells, we tested whether OX40 ligand expression correlates with the number of cell divisions completed (Fig. 2d). Th0 cells expressed high levels of OX40 ligand irrespective of whether they had divided or not. The inhibitory effect of OX40 ligand on Th0 proliferation was demonstrated by the fact that most of the Th0 cells that proliferated were OX40-deficient (Fig. 2a), explaining the 5:1 bias toward OX40-deficient cells found after 6 days in culture. In Th2 cultures on day 3, OX40 ligand expression was absent on all cells irrespective of the number of divisions they had completed. Even after restimulation, Th2 cells did not re-express OX40 ligand in the presence of IL-4 (data not shown).

For Th1 stimulated cells, the levels of OX40 ligand were inversely correlated with division history: the cells that had undergone the greatest numbers of divisions had the lowest levels of OX40 ligand (Fig. 2d). These data are consistent with the idea that OX40-signals at the time of priming put a brake on Ag-driven T cell proliferation.
IL-4 also down-regulates expression of OX40 ligand on activated DCs and B cells but not CD4+CD3- cells

In addition to T cells, in man and mouse, OX40 ligand is expressed on activated DCs and can therefore deliver OX40 signals to T cells during priming (6, 7). To test whether IL-4 had similar effects on OX40 ligand expression by DCs, we added IL-4 to activated DC cultures. Both CD8+ and CD8-CD11c+ DCs expressed detectable levels of OX40 ligand after 24 h in vitro even without additional stimulation, but expression was further augmented by anti-CD40 treatment (Fig. 3, a and b). However, incubation of control or anti-CD40 treated DCs with IL-4 almost completely abrogated expression of OX40 ligand; this was confirmed independently by demonstrating reduced expression of OX40 ligand mRNA in IL-4 treated cultures (data not shown). In contrast, expression of the TNF ligand, CD70, on resting and activated DCs was not significantly affected by incubation with IL-4. CD30 ligand was not detectable on DCs cultured under these conditions.

Recently we reported that the OX40-dependent survival of primed Th2 but not Th1 CD4 T cells was associated with a CD4+CD3- accessory cell located at the B:T interface and in B follicles (Fig. 4a) (5). This accessory cell constitutively expresses high levels of the TNF ligands, OX40 ligand, and CD30 ligand. Unlike T cells and DCs, expression of OX40 ligand on CD4+CD3- cells was not altered by incubation with IL-4, while expression of CD30 ligand was attenuated slightly and TRANCE expression was enhanced (Fig. 3c).

OX40 ligand expression from activated B cells has also been linked with survival of Th2 cells (21). Therefore, we looked at the effects of IL-4 on B cell expression of OX40 ligand (Fig. 3d). As for DCs, IL-4-abrogated CD40-induced expression of OX40 ligand on B cells, whereas expression of 4-1BB ligand and CD70 was enhanced by IL-4.

Discussion

Following activation on DCs, CD4 T cells up-regulate OX40 rapidly, enabling interaction with the ligand expressed either on T cells themselves or on activated DCs. Our data are consistent with a model whereby early OX40-signal acts as a check on T cell proliferation while T cells integrate additional signals from DCs (Fig. 4b). Our data do not suggest a primary role for OX40 signals in directing Th1 or Th2 differentiation. Both Th1 and Th2 effectors down-regulate OX40 ligand signals, but IL-4 abrogates OX40 signals more effectively. As a consequence, Th2 effectors, released from the OX40 brake on proliferation, can out-proliferate their Th1 counterparts. In contrast to Th1 and Th2 cells, Th0 stimulated cells, which failed to differentiate into cytokine-producing effectors, maintained high levels of expression of OX40 ligand, and this was associated with impaired proliferation, analogous to that observed in vivo with peptide immunization (22). We suggest that OX40 signals received during priming, by promoting expression of the Bcl-2 and Bcl-xL proteins that lead to cell cycle exit and survival as opposed to proliferation (4), might be important in preventing the proliferation of T cells stimulated on DCs by innocuous foreign or self protein Ags in vivo in the absence of “adjuvant” signals that promote either Th1 or Th2 responses. This would explain the observation made an in vivo model of diabetes, that OX40-deficient transgenic T cells responding to a self-Ag proliferate more rapidly than normal cells (18).

Although in vitro, exogenous IL-4 is added to induce Th2 differentiation, it is well established that simple alum-precipitated proteins that are used in many common vaccines also selectively induce Th2 rather than Th1 responses (23). The IL-4 produced is derived from T cells (24) suggesting that specific signals during T cell-DC interactions promote Th2 development. We demonstrate here that OX40 signals have a marginal effect on either Th1 or Th2 commitment and there are now more likely candidates for influencing Th2 commitment (25). However, we did find that IL-4, by abrogating OX40 ligand expression on T cells and DCs, removes

FIGURE 3. Expression of TNF family members on CD8+CD11c+ DCs (a), CD8+CD11c- DCs (b), CD4+CD3- cells (c), and B cells (d) after 2-day in vitro culture with activation agents. Results are representative of six separate experiments. Numbers show the percent positive relative to a control Ab staining.

FIGURE 4. Digitally processed confocal microscopes of mouse spleen sections to show the location of DCs and CD4+CD3- cells (a) and a model of distinct functions of OX40 signals during priming on DCs (b and c) and following interaction with CD4+CD3- cells (d). a, Confocal micrographs of mouse spleen sections processed as described in (5) showing IgM-stained cells (gray), CD4+CD3-CD11c- cells (red) and CD11c+ DCs (green). b, In the T zone, naive CD4 T cells are primed on Ag-presenting DCs. c, OX40-signals prime naive CD4 T cells to secrete IL-4, which directs differentiation of T cells into Th2 cells. Once Th2 cells produce IL-4, this down-regulates the expression of OX40 ligand on T cells and DCs, abrogating OX40 signals and allowing rapid T cell proliferation. d, OX40 ligand negative Th2 cells migrate to outer T zone where they receive survival signals through OX40 from CD4+CD3- cells, whose expression of OX40 ligand is not affected by IL-4.
a check on T cell proliferation, allowing IL-4-producing Th2 cells to proliferate rapidly (Fig. 4c). Because OX40 ligand expression is not extinguished on Th0 cells, and to a much lesser extent on Th1 effectors, OX40 signals can significantly influence the ratio of Th1 and Th2 effectors produced in an immune response. This provides a simple explanation of why OX40 blockade enhances Th1 responses and disease resistance in Leishmaniasis (3, 12, 26). In vivo studies of T and B cell responses have shown that Ag-specific T and B cells accumulate at the T:B interface (27), sites to which Th2 cells migrate (28). This is the location for CD4+CD433+ cells (Fig. 4a), which constitutively express high levels of OX40 ligand, and which we have shown are important for the OX40 dependent rescue of Th2 primed cells (Fig. 4d) (5). The expression of OX40 ligand on these cells was not affected by IL-4.

T cells interact with DCs and CD4+CD433+ cells sequentially so that Th2 primed cells proliferating on DCs do not receive OX40 signals until they reached the outer T zone (Fig. 4a). As Th2 cells lack OX40 ligand expression, their continued survival is dependent on OX40 ligand signals from CD4+CD433+ cells, leading to the up-regulation of Bcl-2 and Bcl-xL proteins (4). We suggest that this explains the accumulation of Ag-specific T cells observed at the B:T interface (27, 28), sites where Ag-specific B cells also accumulate (27, 29). This provides an ideal environment for promoting T cell help for B cells for both the extracellular and germinal center responses.

This report provides data that help resolve conflicting concepts on the role of OX40 signals in immune responses. It demonstrates that although OX40 signals might always fundamentally act by up-regulating Bcl-2 and Bcl-xL proteins to promote survival and exit from cell cycle, the downstream effects of these signals depend on timing. Exerted at the time of priming naive cells on DCs they act as a brake on T cell proliferation, perhaps limiting inappropriate T cell responses to self Ags and harmless peptides. In contrast, once Th2 effector differentiation has been initiated, OX40 ligand is down-regulated on T cells and DCs, and this is linked with the rapid proliferation of Th2 committed effectors. Because the down-regulation of OX40 ligand on Th1 effectors is less marked, OX40 signals can still limit their proliferation, thereby affecting the balance of Th1 vs Th2 effectors. Having lost their OX40 ligand expression, Th2 effectors then depend for their survival on signaling through their own OX40 from the OX40 ligand on CD4+CD433+ cells in the outer T zone, which rescues them into the effector/memory pool. This explains the localization of T cells in the outer T zone during the provision of help for B cell responses. The data presented here illustrate how OX40 signals can have different effects on priming and memory responses depending on the site and timing of their application.

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