The OX-40 receptor provides a potent co-stimulatory signal capable of inducing encephalitogenicity in myelin-specific CD4⁺ T cells

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Abstract

The OX-40 receptor, a member of the nerve growth factor/tumor necrosis factor receptor gene family, is expressed preferentially on autoreactive CD4⁺ T cells isolated from the site of inflammation in rats with clinical signs of experimental autoimmune encephalomyelitis (EAE). To examine whether the OX-40 receptor has biologic relevance to T cell function, we evaluated the ability of a rat OX-40 receptor-specific antibody to co-stimulate a myelin basic protein (MBP)-reactive CD4⁺ T cell line. The anti-OX-40 antibody provided a potent co-stimulatory signal to CD4⁺ T cells when added in conjunction with a suboptimal dose of anti-CD3, but the anti-OX-40 antibody alone did not produce a mitogenic response. The magnitude and dose–response of anti-OX-40 co-stimulation was virtually identical to the signal delivered to T cells when cultured with anti-CD28 in conjunction with anti-CD3. MBP-specific T cells stimulated with both anti-CD3 and anti-OX-40 antibodies expressed increased mRNA and protein for IL-2 when compared to anti-CD3 alone. MBP-specific T cells stimulated with both anti-CD3 and anti-OX-40 antibodies were also able to induce EAE when transferred into naïve Lewis rats. In contrast, cells stimulated with anti-CD3 alone were not encephalitogenic. These data suggest that the function of the OX-40 receptor on activated T cells is to provide an alternative pathway for T cell co-stimulation that may be similar in potency to the CD28-mediated signal.

Introduction

At least two signals are necessary to activate a CD8 or CD4 T cell response to antigen (1). The first signal is delivered through the TCR by antigen (peptide) bound to MHC class I or II. The best characterized second signal is delivered via an interaction between the CD28 receptor on the T cell and its ligands B7-1 or B7-2 on the antigen-presenting cell (APC). This interaction stimulates the production of IL-2 and IFN-γ, which are cytokines involved in the development of a T cell response. Once T cells are activated, another receptor is expressed, CTLA-4, which is homologous to CD28 and binds B7 molecules with a higher affinity than CD28. The interaction between B7 and CTLA-4 inhibits the activation signal of CD28 and delivers a negative signal that may prevent T cell responses (2,3). As such, alternative molecules may be required to co-stimulate naive T cells (4–7) or to maintain T cell activation in cases where immune reactivity needs to be sustained. In this work we explore the magnitude and dose–response of signals delivered via a new co-stimulatory molecule, the OX-40 receptor.

The OX-40 receptor is expressed primarily on activated CD4⁺ T cells (8) and has been described as a lymphocyte-specific member of a growing family of receptors for membrane-bound and soluble cytokines, termed the tumor necrosis factor (TNF) receptor (R) superfamily (9–10). The TNF-
CD40, Fas/APO-1 (CD95), lymphotoxin-1 (TNF-R family includes the two TNF-R, the CD30 antigen, CD27, and 4-1BB, all of which are expressed on cells of hematopoietic lineage. These membrane-bound proteins are characterized by the specific spacing of 13–15 cysteine residues in the extracellular N-terminal domain, which creates a tertiary structure thought to be involved in ligand binding. A common function of the TNF-R superfamily seems to be in the regulation of activation and/or apoptosis of lymphocytes (11–13). For instance, the signal transmitted through CD40 is pivotal for Ig isotype switching and prevents programmed cell death of germinal center B cells (11). In contrast, signaling through the Fas/APO-1 antigen induces apoptosis (12,13) and lack of a functional Fas protein leads to a generalized autoimmune syndrome that may result from impaired deletion of autoreactive lymphocytes (13). Therefore, there appear to be distinct cellular and molecular mechanisms underlying the physiological responses that are mediated by members of the TNF-R superfamily.

The mouse, rat, and human forms of the OX-40 receptor have been cloned and sequenced (10,14,15). Its homology to an important family of cell surface receptor genes (TNF-R) and the restricted expression on activated CD4+ T cells suggests that the OX-40 receptor may play an important role in immune cell activation and regulation. The OX-40 receptor has recently been shown to be expressed selectively on T cells isolated from the site of inflammation in a number of disease states including experimental autoimmune encephalomyelitis (EAE) (16,17), rheumatoid arthritis (17), graft-versus-host disease (18) and on tumor-infiltrating lymphocytes (19). The importance of OX-40 receptor expression in EAE was demonstrated recently by the observation that an anti-OX-40 antibody could selectively identify the pathogenic T cells isolated from the inflammatory sites in the target organ (spinal cord or brain). The OX-40 receptor+ T cells isolated from these sites exhibit TCR-CD3 binding motifs specific for autoantigen reactivity (20) and produced pro-inflammatory cytokines (IL-2 and IFN-γ) at the time of isolation (16). Additionally, we reported that MBP-reactive CD4+ T cells that induce EAE predominantly utilize the Vβ8.2 TCR and express the OX-40 receptor when stimulated in vitro with antigen (21,22). Taken together, these findings indicate that the OX-40 antibody could be used as a tool for studying autoantigen-specific T cells without prior knowledge of the autoantigen.

To develop a system for studying the mechanisms of T cell effector function in EAE, our laboratory has generated myelin basic protein (MBP)-specific, lymph node-derived T cell lines that respond to concanavalin A (Con A), whole MBP and the major encephalitogenic epitope (residues 72–89) of MBP (23). These lines are usually >95% CD4+ 30–50% Vβ8.2+ and 100% of the cells express the OX-40 receptor upon activation, as determined by FACS analysis (data not shown). Moreover, when stimulated with antigen (MBP) and APC, the lines induced clinical signs of EAE within 6 days following transfer of cells into naive Lewis rats (23). In this report, we have identified a role of the OX-40 receptor in CD4+ T cell co-stimulation. We have found that cross-linking the OX-40 receptor on anti-CD3 stimulated cells generates an anti-OX-40 dose-dependent mitogenic signal that is similar in potency to CD28 co-stimulation. Furthermore, cells stimulated with both anti-CD3 and anti-OX-40 produce enhanced levels of IL-2, and were capable of inducing clinical signs of encephalomyelitis when transferred to naive Lewis rats. Collectively, our results support a co-stimulatory capacity attributable to rat OX-40 receptor signaling within antigen-specific CD4+ T cells, and suggest that during inflammatory responses in vivo it may provide a pathway for T cell activation that is distinct from the classic CD28-based co-stimulation.

Methods

Animals and cell lines

Lewis rats were obtained from the NIH (Bethesda, MD) and were housed at the VA Medical Center Animal Facility (Portland, OR). Lymph node-derived MBP-reactive T lymphocyte lines (23) were re-stimulated in vitro with MBP (20 µg/ml) presented by irradiated Lewis rat thymocytes in RPMI and 1% rat serum (stimulation media) for 3 days at 37°C. The cells were expanded further in RPMI supplemented with recombinant IL-2 (100 U/ml) and 10% FCS (growth media) for 7–10 days before reactivation.

Proliferation assays

After expansion in growth media, MBP-specific T cells were harvested, washed, counted and resuspended in stimulation media for use in a proliferation assay as previously described (23). For activation, 2 × 10^5 T cells were stimulated in 96-well flat-bottom plates for 48 h in stimulation media and pulsed for 18 h with 1 µCi [3H]thymidine. The cells were harvested and mean thymidine incorporation (c.p.m.) was calculated from triplicate wells.

mAb

mAb to rat CD3, OX-40 and CD28 were commercially obtained from PharMingen (La Jolla, CA).

Induction of passive EAE

Bulk activation of T cell lines for passive transfer of EAE was carried out in six-well plates (Costar, Auburn, WA), starting with 2 × 10^6 T cells cultured with either plate-bound anti-CD3 alone or plate-bound anti-CD3 with soluble anti-OX-40. After 3 days of culture, the T cell blasts were washed, counted, resuspended in RPMI and injected i.p. into naive Lewis rats. The recipient rats were inspected daily following injection and the clinical signs of disease were scored as follows: 0 = no signs; 1 = flaccid tail; 2 = ataxia; 3 = hind quarter paralysis; 4 = quadriplegic or moribund.

Cytokine analysis by PCR

T cells were seeded at 2 × 10^6/ml in 24-well flat-bottom plates and cultured in the presence of plate-bound anti-CD3 with and without anti-OX-40, antigen (MBP) plus APC or with APC alone. After 8, 14 and 24 h of culture, cells were harvested, counted and immediately lysed using the RNA-STAT-60 buffer (TEL TEST, Friendswood, TX). RNA from equal numbers of cells was synthesized into cDNA with MLV RT (Gibco) and the cDNA was used as a template for primed amplification of the indicated cytokine messages using published primers (16). To normalize for amount of cDNA used for each sample,
the primer pairs for the enzymatic reporter gene GAPDH were utilized in a 35 µl reaction containing 1 µl of cDNA and run for 27 cycles. Amplification of IL-2 and IFN-γ message was done for 35 cycles. The PCR products were resolved and visualized on ethidium bromide-stained 1.5% agarose gels and displayed as one band of the appropriate mol. wt for each primer set.

ELISA
T cells were seeded at $2 \times 10^6$/ml in 24-well flat-bottom plates and cultured in the presence of plate-bound anti-CD3 with or without soluble anti-OX-40, antigen (MBP) plus APC or with APC alone. After 14 and 24 h of stimulation, 2 ml of supernatants from each set were collected and stored at −70°C until they were used for assessment of IL-2 by standard ELISA (PharMingen).

Results and discussion
We reported previously that encephalitogenic CD4+ T cells up-regulate the OX-40 receptor when stimulated in vitro with MBP and APC (21,22). Therefore, using a lymph node-derived CD4+ T cell line specific for MBP, we have explored the ability of the OX-40 receptor to transduce a co-stimulatory signal by stimulating T cells with anti-CD3 alone or in conjunction with anti-OX-40 mAb. The MBP-reactive line used in this study was >95% CD4+, >50% Vβ8.2+ and 100% of the cells expressed the OX-40 receptor upon activation. Figure 1(A) shows T cell proliferation in the presence of a constant amount of anti-OX-40 mAb (10 µg/ml) while adding increasing concentrations of anti-CD3. At each dose of anti-CD3 examined, the addition of anti-OX-40 resulted in enhanced T cell proliferation. Anti-OX-40 alone did not induce proliferation of T cells (Fig. 1B). It should be noted that up to 10 µg/ml of anti-CD3 was regarded as submitogenic since, in the absence of anti-OX-40 mAb, T cell proliferation at this concentration was not significantly different from the lowest concentration of anti-CD3 (0.5 µg/ml) that we tested. Anti-CD3 (10 µg/ml) produced the highest proliferative response in conjunction with anti-OX-40 and this concentration of anti-CD3 was used for all subsequent experiments.

It is well established that Th1 cell activation and proliferation are accompanied by synthesis and production of IL-2 (24); therefore, we sought to determine whether the mechanism for the co-stimulatory effect of the anti-OX-40 antibody (Fig. 1A) involved production or responsiveness to IL-2. Figure 1(B) represents an experiment where MBP-specific T cells were cultured with anti-CD3, anti-OX-40, recombinant IL-2 or combinations of these reagents as indicated. The data show that the T cells exhibited some responsiveness to recombinant IL-2 alone, this was not surprising since FACS analysis prior to T cell activation showed moderate levels of the IL-2 receptor α chain (data not shown). Anti-OX-40 antibody alone did not promote T cell proliferation. When cells where cultured with both IL-2 and anti-CD3, there was >3-fold enhancement over the effect of IL-2 alone and >5-fold over control untreated cells, implying that the cells became more responsive to IL-2 after anti-CD3 stimulation. These results are consistent with the idea that anti-CD3 up-regulates IL-2 receptor expression, which would allow the cells to become more responsive to recombinant IL-2. These results support a model whereby cross-linking both the TCR and OX-40 receptor may lead to production of IL-2, which in turn would be responsible for enhanced T cell proliferation.

To test the possibility that OX-40 receptor cross-linking in combination with anti-CD3 is sufficient to co-stimulate T cell proliferation, MBP-specific T cells were stimulated with a constant amount of either soluble or plate-bound anti-CD3 (10 µg/ml) in the presence of increasing amounts of the anti-OX-40 antibody. As shown in Fig. 2, plate-bound anti-CD3 was more efficient at generating the first activation signal and the anti-OX-40 antibody elicited a dose-dependent increase in T cell proliferation with stimulation index of 90-fold. This suggests that OX-40 receptor function may be coupled to
Fig. 2. OX-40 dose-dependent co-stimulation of anti-CD3-stimulated MBP specific CD4+ T cells. T cells were seeded in a 96-well flat-bottom well at 2 x 10^5/well and stimulated with 10 μg/ml of either soluble or plate-bound anti-CD3 plus increasing concentrations of anti-OX-40 antibody. The cells were cultured for 48 h, labeled with [3H]thymidine for 18 h, and were then harvested and counted. Results are presented as mean c.p.m. with SD calculated from triplicate wells.

TCR ligation, and is directly linked to T cell proliferation. We propose that in vivo, this TCR–OX-40 receptor coupling would be initiated by T cell recognition of antigen (e.g. MBP in EAE) via the TCR, accompanied by co-stimulation via OX-40 receptor interaction with its ligand on an APC within the inflammatory compartment. Such an interaction would lead to proliferation and secretion of pro-inflammatory cytokines by the pathogenic CD4+ T cells as previously reported (9, 14), resulting in histologic lesions in the central nervous system.

To test the hypothesis described above we analyzed the profile of cytokine mRNA expression in MBP-specific cells stimulated with anti-CD3 alone or in conjunction with anti-OX-40. As shown in Fig. 3(A), RT-PCR revealed that anti-CD3-stimulated cells expressed high levels of IFN-γ mRNA, but IL-2 mRNA was not detected. When T cells were cultured with anti-CD3 and anti-OX-40, both IL-2 and IFN-γ mRNA were detectable at levels comparable to those observed in cells stimulated with antigen (MBP) and APC. For the most part cytokine mRNA was not detected in APC alone, although a faint IFN-γ band was apparent. GAPDH PCR primers were used to confirm that a similar amount of cDNA template was used for all samples. PCR analysis was performed with cells stimulated for 8 h, because the earlier time point showed the highest level of cytokine mRNA production compared to 14 and 24 h. IL-2 protein levels in the supernatants harvested from the same cells used for PCR were measured by ELISA.

There was a positive correlation between IL-2 secreted into the culture medium and the profile of mRNA expression in each case (Fig. 3B). A proliferation assay further confirmed that the same cells used in Fig. 3(A and B) maintained responsiveness to Con A, antigen and the dose-dependent co-stimulatory signal delivered through the OX-40 receptor (Fig. 3C). We noticed, however, that while no IL-2 mRNA was detectable in cells stimulated with anti-CD3 alone (Fig. 3A), IL-2 protein was detected (150 pg/ml) in the supernatant (Fig. 3B). One explanation could be that although there was some cellular synthesis of IL-2, the level of IL-2 mRNA in the cells stimulated with anti-CD3 alone was too low to be detected after 35 cycles of PCR amplification. However, since these cells were not proliferating (Fig. 3C) they may not have up-regulated the IL-2 receptor and the small amount of secreted IL-2 was not internalized by the T cells and therefore was detected by our ELISA assay. In contrast, cells stimulated with both anti-CD3 and anti-OX-40 were actively synthesizing detectable IL-2 mRNA while at the same time consuming the secreted IL-2 to sustain their proliferation. The results suggest that either the requirements of antigen-primed T cells for the production of IL-2 and IFN-γ may be distinct, as previously reported (25), or that IFN-γ mRNA may have pre-existed in the cells before culture, perhaps as an inherent property of the autoreactive MBP-specific T cells. We tend to favor the initial hypothesis because the levels of IFN-γ mRNA appeared to be the similar in both the anti-CD3, anti-CD3 and anti-OX-40 stimulated cells. The data also illustrate the ability of cross-linked OX-40 receptor to potentiate the activation and biologic function of T cells through 'T cell growth factor' (IL-2).

We next determined whether OX-40 receptor function might be similar in potency to the classic B7/CD28 system of co-stimulation (1). CD28 cross-linking has been shown to be important for the generation of many biological responses, including cytokine production (26), cytotoxic T lymphocyte generation (27), allograft and xenograft rejection (28,29), antibody production (30), and tumor surveillance (27,31). However, the B7/CD28 co-stimulatory system is becoming highly complex as more molecules are identified, such as CTLA-4, that can counteract the function of CD28 on T cells (1,3). The existence of such counteractive molecules should warrant the requirement for alternative co-stimulatory pathways in which the OX-40 receptor may play a role. Therefore, we compared the magnitude of co-stimulation by the OX-40 receptor to that delivered through CD28, by cross-linking anti-CD3-stimulated cells with either anti-OX-40 or anti-CD28. Figure 4 shows that T cells cultured with anti-CD3 and increasing amounts of anti-OX-40 antibody exhibited virtually the same magnitude and dose–response of T cell proliferation as the same cells cultured with anti-CD3 and anti-CD28. This observation supports our hypothesis that the OX-40 receptor–ligand interaction could provide an alternative, CD28-independent co-stimulatory pathway to drive antigen-specific T cells to proliferate. Thus, in cases where immune reactivity ought to be sustained, such as in anti-tumor and anti-viral immunity, the interaction of OX-40 with its ligand may provide the required alternative signal to maintain the survival of immune-reactive T cells, ultimately generating an antigen-specific T cell memory population. We are investigating this possibility in future studies.

It has previously been shown that MBP-specific T cells need to be activated in vitro for 48–72 h before they can initiate clinical signs of EAE upon adoptive transfer (23). Therefore, to determine whether the signal generated by the OX-40 receptor is sufficient to activate the biologic function of disease-associated T cells, we examined whether the MBP-
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Fig. 3. OX-40-induced proliferation is related to transcription and secretion of IL-2. (A) RT-PCR amplification of IL-2 and IFN-\(\gamma\) mRNA message after stimulation. MBP-specific T cells were seeded at 2 \(\times\) 10\(^6\) ml in 24-well flat-bottom plates and stimulated as indicated in Methods. After 8 h of culture, cells were harvested, counted and immediately lysed. Total RNA from 1 \(\times\) 10\(^6\) cells was synthesized into cDNA and amplified with the appropriate primers for 27 cycles (GAPDH) or 35 cycles (IL-2 and IFN-\(\gamma\)). The PCR products were visualized on ethidium bromide-stained 1.5% agarose gels. (B) ELISA detection of IL-2 protein secretion after stimulation of the MBP-specific T cells with anti-CD3 with or without anti-OX-40. MBP-specific T cells were seeded at 2 \(\times\) 10\(^6\) ml in 24-well flat-bottom plates and cultured in the presence of plate-bound anti-CD3 with or without anti-OX-40, antigen (MBP) plus APC, or with APC alone as described in (A) above. After 14 and 24 h of culture, supernatants were harvested and analyzed for IL-2 content by standard ELISA. IL-2 was quantitated by comparison with standard curves generated using recombinant IL-2 standards and expressed as pg/ml. Data represent the mean and SD of triplicate wells, \(P < 0.05\) by Student’s t-test. (C) Proliferation analysis of MBP-specific T cells. Cells were seeded as indicated in the legend of Fig. 1 and cultured with Con A, antigen plus APC and anti-CD3 (10\(\mu\)g/ml) alone or in the presence of increasing concentrations of anti-OX-40 as indicated. The cells were cultured, labeled with \(^{3}H\)thymidine and thymidine uptake was analyzed as described in Fig. 1.

specific T cell lines could transfer EAE after bulk stimulation with anti-CD3 and anti-OX-40. Figure 5 shows the results of two experiments, where animals injected with cells that were pre-stimulated with both anti-CD3 and anti-OX-40 antibodies developed EAE. In contrast, animals that were injected with the same number of cells that were pre-stimulated with anti-CD3 alone did not show clinical signs of disease (data not shown). These observations demonstrate that activation of CD4\(^+\) T cell effector function can occur through OX-40 receptor mediated co-stimulation.

A T cell co-stimulatory role for some other members of the TNF-R family has been described in other systems. For instance, it has been shown that the murine 4-1BB receptor (32), also an inducible T cell activation antigen, can transmit a co-stimulatory signal to T cells when cultured with anti-4-1BB in conjunction with anti-CD3 (33,34). Moreover, a soluble
in vivo injection of a soluble chimeric OX-40 receptor molecule on accessory cells. Similarly, we have recently found that block may be at the level of expression of the 4-1BB ligand found to block proliferation as well as cytokine production by chimeric molecule of 4-1BB receptor (4-1BB:IgFc) was also seeded in a 96-well flat-bottom plate at $2 \times 10^5$/well and stimulated with 10 µg/ml of plate-bound anti-CD3 in the presence of increasing amounts of either anti-OX-40 or anti-CD28. The cells were cultured with the antibodies for 48 h, labeled with [3H]thymidine for 18 h, and were then harvested and counted. Results are presented as mean c.p.m. with SD calculated from triplicate wells.

chimeric molecule of 4-1BB receptor (4-1BB:IgFc) was also found to block proliferation as well as cytokine production by anti-CD3-stimulated, splenic T cells (35), suggesting that the block may be at the level of expression of the 4-1BB ligand on accessory cells. Similarly, we have recently found that in vivo injection of a soluble chimeric OX-40 receptor molecule (OX-40:human IgFc) was also able to inhibit clinical signs of EAE (manuscript in preparation). Thus, we believe that the inhibition by soluble OX-40 in our system may also be occurring by blocking OX-40 ligand-mediated co-stimulation between APC presenting autoantigen to myelin-specific T cells in the CNS. The actual APC responsible for activating T cells during EAE remains to be defined; however, we have recently found a cell population in the CNS of animals with EAE that is OX-40 ligand $+/Mac-1^-/MHC$ class II $^+$ and we are currently assessing whether this cell type can present autoantigen to myelin-reactive T cells in vitro.

The current data suggest that a signal generated through the OX-40 receptor induces T cell co-stimulation in a manner very similar to that generated through CD28. The OX-40 receptor may be functioning via a mechanism observed in B cell activation, where interactions between CD40 and its ligand play a central role in the reciprocal communication between T and B cells that leads to B cell differentiation and survival (36). CD40–CD40L interaction controls co-stimulatory activity of B cells by inducing the expression of B7-1 or B7-2 and other molecules, an event that subsequently directs T cell activation and development of effector functions (36). It has been shown that mAb to CD40 (37) or CD40L (38) inhibit the induction of B7-1 and B7-2 expression during T–B cell communication. Moreover, a mAb that is involved in the CD40L-induced co-stimulatory activity in CD28-deficient mice does not have any reactivity with B7-1 or B7-2 (39), suggesting that CD40 could also be functioning as part of a novel co-stimulatory pathway. We suggest, based on these parallel systems, that the OX-40 receptor–ligand interaction is yet another means by which activated T cells can be driven to proliferate and perhaps sustain effector function in a CD28-independent manner. Future studies will examine the role of OX-40 in CD28-deficient animals, where co-stimulatory requirements for T cell function may be modified, as previously observed (40,41).

The results we have described in this paper may have several implications for a number of disease states where CD4$^+$ T cells have been found to be chronically stimulated, such as autoimmunity (16,17), graft versus host disease (18) and solid tumors (19). In each of these cases, the CD4$^+$ T cells that expressed the OX-40 receptor also co-expressed 'memory' markers such as CD45RO (17) and high levels of CD44 (data not shown). Recently, we have transfected MHC class II$^+$ fibroblasts (25) with the OX-40 ligand in order to determine at which stage of T cell development the OX-40 receptor was biologically functional. Preliminary results suggest that OX-40 receptor signaling is important during the 'effector' T cell stage. Notably, little or no OX-40 receptor-specific co-stimulation was observed when priming a 'naive' T cell population, even though the OX-40 receptor was expressed within 24 h of naive T cell activation (M. Croft, pers. commun.). Therefore, we hypothesize that signaling through the OX-40 receptor may correspond to a memory or effector population rather than a naive T cell encountering antigen for the first time.

Ongoing studies are aimed at defining the physiological trigger for OX-40 receptor expression and signaling, as well as the molecular determinants for its regulation in antigen-specific memory T cell populations. Of particular interest is a determination of the intracellular biochemical events, such as interactions between the OX-40 receptor and cytosolic receptor-associated proteins. It is known that cross-linking CD40 on B cells increases cellular tyrosine phosphorylation (42) and controls B cell differentiation. Additionally, the low- and high-affinity NGF receptors associate with the pro-oncogene trk (43), a protein tyrosine kinase, indicating that members of the TNF/NGF-R family of proteins can be part of the regulated receptor complexes that function in signaling. OX-40 receptor regulation may also occur at the level of TCR-mediated antigen reactivity, or other surface molecules up-regulated on T cells following antigen recognition.

In conclusion, our findings affirm a role for co-stimulation mediated through the OX-40 receptor. We have found that cross-linking of the OX-40 receptor on anti-CD3 stimulated T cells can result in $>90$-fold enhancement of T cell proliferation over the effect of anti-CD3 alone. These results imply that the OX-40 receptor is an inducible molecule that contributes to T cell co-stimulation. Its expression on activated T cells in vivo may allow for the interaction with its ligand. This interaction would cause IL-2 production by antigen-specific CD4$^+$ T cells, resulting in T cell stimulation/
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Fig. 5. OX-40 cross-linking induces encephalitogenicity in anti-CD3-stimulated MBP specific T cells. T cells were seeded in six-well plates at $2 \times 10^6$/well and stimulated with 10 $\mu$g/ml of plate-bound anti-CD3 with or without (control) 20 $\mu$g/ml of anti-OX-40. 3 $\times 10^6$ (rats 1 and 2) or 4.5 $\times 10^6$ (rats 3–5) T cell blasts were suspended in 1 ml of RPMI and injected i.p. into naive Lewis rats. Control animals (not shown here) were injected with either 3 $\times 10^6$ ($n = 3$) or 4.5 $\times 10^6$ ($n = 3$) T cells stimulated with plate-bound anti-CD3 antibody only and never showed clinical signs of disease. After transfer, animals were monitored daily for clinical signs of disease according to: 0 = no signs, 1 = limp tail, 2 = hind limb weakness, 3 = hind quarter paralysis, 4 = moribund or dead.

proliferation within inflammatory lesions. We predict that this OX-40-mediated response may be similar to that generated by the interactions between CD40–CD40L or CD27–CD70, both members of the TNF-R family of molecules. As discussed both here and in other studies (33–34,44), our results support the notion that activated T cells express an evolving array of cell membrane-bound molecules that are intimately involved in immune modulation. Future studies will explore the cellular and molecular mechanisms involved in OX-40 receptor expression and co-stimulation in order to identify alternative approaches to therapeutic intervention in certain disease states, where the etiology and progression of disease involves selective display of the OX-40 receptor.
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Abbreviations

- APC: antigen-presenting cell
- Con A: concanavalin A
- EAE: experimental autoimmune encephalomyelitis
- MBP: myelin basic protein
- NF: nerve growth factor
- R: receptor
- TNF: tumor necrosis factor

References


