OX40 Ligand and CD30 Ligand Are Expressed on Adult but Not Neonatal CD4+CD3− Inducer Cells: Evidence That IL-7 Signals Regulate CD30 Ligand but Not OX40 Ligand Expression

Mi-Yeon Kim, Graham Anderson, Andrea White, Eric Jenkinson, Wiebke Arlt, Inga-Lill Martensson, Lena Erlandsson and Peter J. L. Lane


http://www.jimmunol.org/content/174/11/6686

References

This article cites 28 articles, 8 of which you can access for free at: http://www.jimmunol.org/content/174/11/6686.full#ref-list-1

Subscription

Information about subscribing to The Journal of Immunology is online at: http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
OX40 Ligand and CD30 Ligand Are Expressed on Adult but Not Neonatal CD4⁺CD3⁻ Inducer Cells: Evidence That IL-7 Signals Regulate CD30 Ligand but Not OX40 Ligand Expression

Mi-Yeon Kim,* Graham Anderson,* Andrea White,* Eric Jenkinson,* Wiebke Arlt,† Inga-Lill Martensson,‡ Lena Erlandsson,‡ and Peter J. L. Lane²*

In this report, we have examined the expression of the T cell survival signals, OX40 ligand (OX40L) and CD30 ligand (CD30L) on CD4⁺CD3⁻CD11c⁺B220⁻IL-7Rα⁺ inducer cells from birth to adulthood in mice. We found that adult but not neonatal inducer cells expressed high levels of OX40L and CD30L, whereas their expression of TNF-related activation-induced cytokine (TRANCE) and receptor activator of NF-κB (RANK) was comparable. The failure of neonatal inducer cells to express the ligands that rescue T cells helps to explain why exposure to Ag in neonatal life induces tolerance rather than immunity. The expression of OX40L and CD30L on inducer cells increased gradually in the first few weeks of life achieving essentially normal levels around the time mice were weaned. We found that IL-7 signaling through the common cytokine receptor γ-chain was critical for the optimal expression of both TNF-related activation-induced cytokine and CD30L but not OX40L. Furthermore, glucocorticoids, which potently suppress T effector function, did not influence the expression of OX40L and CD30L in the presence of IL-7. The Journal of Immunology, 2005, 174: 6686–6691.

The capacity of animals to mount high affinity long-lived Ab responses is associated with the development of segregated B and T cell areas in secondary lymphoid tissue and also the development of lymph nodes. Recent work has identified a key role for a CD4⁺CD3⁺CD11c⁺B220⁻IL-7Rα⁺ cell (inducer cell) in this process (1). These cells are found in neonatal lymph nodes before they are colonized by lymphocytes (2). They are one of only a few cell types that express lymphotoxin (LT)αβ (LTαβ) (2), which is the ligand for the LTβ receptor that plays a critical role in organizing secondary lymphoid tissues (3, 4). Mice deficient in this signaling pathway lack lymph nodes, and the spleen is not organized into B and T cell areas. Evidence that LTβ receptor signals from inducer cells initiate a sequence of events that leads to the development of lymph nodes comes from mice deficient in these cells. Inducer cells express IL-7 receptor α chain (IL-7Rα), common cytokine receptor γ-chain (γc chain, CD132), RORγ (retinoic acid receptor-related orphan nuclear hormone receptor), CXCR5, TNF-related activation-induced cytokine (TRANCE), and receptor activator of NF-κB (RANK). Mice deficient in RORγ (5) lack lymph nodes and Peyer’s patches; mice deficient in TRANCE (6) and RANK (7) signals lack most lymph nodes; mice deficient in IL-7 (8) and γc chain signals (9) lack Peyer’s patch anlagen and some lymph nodes; and CXCR5-deficient mice have impaired Peyer’s patch formation and are deficient in some lymph nodes (10).

In adult mice, we identified the adult equivalent of inducer cells, and reported that they were located in B folicles and at the interface between the B and T cell areas (11). These cells had a very similar phenotype to those described in the neonate (11), but in addition to the expression of LTαβ2 and TRANCE, these cells were also found to express high levels of the TNF ligands for OX40 and CD30. We found evidence for direct interactions between primed CD4 T cells and these cells during the course of immune responses, and showed that the survival of primed T cells was partially dependent on OX40 signals from these cells. Because OX40 and CD30 are genetically linked in human (chromosome 1) and mouse (chromosome 4) (12) and share common signaling pathways, it seemed likely that there were redundant signaling pathways, it seemed likely that there were redundant signaling pathways (13). Recently, we have generated and examined the phenotype of mice deficient in both OX40 and CD30 and shown that they lack T cell memory for Ab production, and this failure results from deficient OX40 and CD30 signals delivered by inducer cells (14).

In this study, we have investigated the expression of OX40 ligand (OX40L) and CD30 ligand (CD30L) during the development of inducer cells from the neonatal period to adulthood. We found that inducer cells isolated from neonatal mice did not express OX40L and CD30L, whereas their expression of TRANCE and RANK was similar to adult mice. This suggested that there were specific signals that induced expression of OX40L and CD30L on inducer cells. We found that mice deficient in either IL-7 signals or γc chain had substantially reduced CD30L expression, whereas OX40L expression was comparable. Furthermore, addition of IL-7...
to neonatal inducer cells specifically up-regulated CD30L expression. The IL-7-induced CD30L expression was also observed on memory phenotype CD4 and CD8 T cells as well as naive CD8 T cells, suggesting that there is a common IL-7-dependent pathway for CD30L expression. We failed to identify specific signals that up-regulated OX40L expression on neonatal inducer cells. The expression of OX40L and CD30L was not dependent on MHC class I or II signals or T cells. Furthermore, we show that although glucocorticoids down-regulated CD30L and TRANCE expression on inducer cells, OX40L expression was only modestly affected. However, in the presence of IL-7, CD30L and TRANCE expression was restored on glucocorticoid-treated cells.

**Materials and Methods**

**Mice**

Normal, Rag1 deficient (Rag1<sup>−/−</sup>), and OX40 and CD30 double deficient (OX40<sup>−/−</sup> and CD30<sup>−/−</sup>) C57BL/6 mice were bred and maintained in our animal facility. Rag1<sup>−/−</sup> mice were used as a source of inducer cells (Figs. 1, 4A, and 6). CD3ε transgenic (Tg) 26 mice that lack CD3<sup>+</sup> T cells (15) were obtained from The Jackson Laboratory, and MHC class II-deficient and MHC class I and II double deficient mice (16) were obtained from Taconic Farms. Spleens from IL-7-deficient mice were provided by I.-L. Martensson (Babraham Institute, Cambridge, UK), and spleens from RAG2<sup>−/−</sup> mice deficient in γ chain and IL-7Rα-deficient mice were provided by R. Zamoyska (National Institute for Medical Research, London, UK).

**Preparation of inducer cells**

Cell suspensions for isolation of inducer cells were made from the spleens as follows. Spleens were cut into small fragments and then cultured with Collagenase D (1 mg/ml) (Roche Diagnostics) 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 per the manufacturer’s instructions. CD11c<sup>+</sup> cells were depleted with MACS anti-mouse CD11c microbeads (Miltenyi Biotec) using autoMACS (Miltenyi Biotec). CD4<sup>+</sup> cells from CD11c<sup>+</sup> cells were enriched with MACS anti-mouse CD4 microbeads and positively selected. The resulting populations were cultured overnight. CD4<sup>+</sup>CD3<sup>+</sup>B220<sup>−</sup> cells from CD4-enriched population were stained with appropriate Abs and analyzed by flow cytometry.

**In vitro culture with IL-7 or steroid hormones**

T cells from normal mouse spleen and inducer cells from RAG-deficient mouse spleen were cultured with 100 ng/ml IL-7 (PeproTech) and/or with 100 nM endogenous human glucocorticoid cortisol (= hydrocortisone)
Results

Differential expression of OX40L and CD30L on CD4+CD3− inducer cells from neonatal to adulthood

Adult inducer cells are unusual in that they show constitutive expression of high levels of the TNF ligands for OX40 and CD30 that are expressed on activated T cells (11). However, early in neonatal life these cells play a key role in the development and organization of secondary lymphoid tissue (1). At this stage of development, exposure to foreign Ags is more likely to tolerize than immunize the murine immune system. We were interested to know at what stage in ontogeny the expression of OX40L and CD30L was first found that the numbers of inducer cells isolated from mice deficient in IL-7 and γc chain deficiency (Fig. 2). In contrast, the expression of TRANCE and CD30L was observed on inducer cells isolated from wild-type neonatal and adult mice.

IL-7 and γc chain signaling regulates CD30L but not OX40L expression on inducer cells

Because inducer cells express IL-7RN and γc chain, we first tested whether signaling through these pathways regulated CD30L or OX40L expression. To compare the expression directly, inducer cells were isolated from control and deficient mice and stained with the same concentrations of Abs on the same day. Analysis of IL-7- and IL-7RN-deficient mice showed that OX40L expression was essentially unaffected and this was also true of RAG−/− mice with concomitant γc chain deficiency (Fig. 2). In contrast, the expression of TRANCE but not its receptor, RANK, was impaired in IL-7-, IL-7RN-, and γc chain-deficient mice, and there was also substantially reduced expression of CD30L. This suggested that IL-7 signals up-regulated TRANCE and CD30L expression. We also found that the numbers of inducer cells isolated from mice deficient in IL-7 and γc chain signals was greatly reduced (Fig. 3).

To test directly whether IL-7-induced CD30L expression, inducer cells from neonatal and adult mice were cultured in vitro isolated at the same time from control adult RAG-deficient mice were used to compare levels of expression. It is technically difficult to do this because primed T cells associate with inducer cells. Nevertheless, the same pattern of expression of TRANCE, RANK, OX40L, and CD30L was observed on inducer cells isolated from wild-type neonatal and adult mice.

FIGURE 2. Expression of IL-7RN, γc chain, TRANCE, RANK, OX40L, and CD30L on inducer cells isolated from IL-7-deficient, IL-7RN-deficient, and RAG−/− γc chain-deficient mice compared with control RAG−/− mice. Results are representative of two separate experiments. To compare levels of expression, inducer cells were isolated from the various knockout and control mice contemporaneously. Closed histograms are control staining with biotinylated Abs.

IL7Rα γc chain

TRANCE RANK OX40L CD30L

RAG−/−

FIGURE 3. Numbers of inducer cells isolated from the spleens of gene-deficient mice. Results are representative of at least two separate experiments. Bars show SD between experiments.
with 1–100 ng/ml IL-7 (data shown of 100 ng/ml IL-7) for 6 days (Fig. 4A). Compared with freshly isolated or untreated cells, IL-7 clearly induced CD30L expression on neonatal inducer cells and enhanced CD30L expression by adult inducer cells (Fig. 4A). IL-7 also clearly up-regulated TRANCE expression but OX40L expression was unaffected. This effect of IL-7 was not attributable to selective survival of inducer cells expressing high levels of TRANCE and CD30L, because the viability and cell number of inducer cells from IL-7 treated and control cultures were similar (data not shown).

The induction of CD30L by IL-7 was not restricted to inducer cells. Both CD44high populations of CD4 and CD8 T cells up-regulated CD30L expression in response to IL-7 (Fig. 4B), and this was also true for CD44low CD8 naive T cells (data not shown). Whereas IL-7 augmented CD30L expression, TRANCE or OX40L expression was not induced on T cells by IL-7.

IL-7 signals up-regulated CD30L and TRANCE expression, and this was also true for IL-2 (data not shown). This suggests that this signal was mediated by \( \gamma_c \) chain. In contrast, other cytokines that signal through \( \gamma_c \) chain (17), IL-4, IL-9, and IL-15, had little effect on the expression of OX40L, CD30L, or TRANCE (data not shown).

**MHC class I and II expression does not affect OX40L and CD30L expression**

Because inducer cells express CD4, we tested whether interaction with MHC class I and class II molecules were key to regulating expression of OX40L and CD30L. This was not the case although OX40L and CD30L expression was slightly lower in mice deficient in MHC class I and II molecules (Fig. 5). Also CD3+ T cells did not influence inducer cell development as mice lacking T cells, but normal numbers of B cells (15) showed the highest levels of expression of OX40L and CD30L (Fig. 5), and the numbers of inducer cells isolated per spleen was consistently augmented compared with RAG−−/− mice (Fig. 3). There is some evidence for reverse signaling through TNF ligands (18, 19) but there was no evidence that this was important for inducer cells. Mice deficient in OX40 and CD30 had normal numbers (data not shown), and their expression of TRANCE, RANK, OX40L, and CD30L was also normal (Fig. 5).

![Effects of glucocorticoids on TNF expression on inducer cells](http://www.jimmunol.org/)

Because glucocorticoids are commonly used to suppress immune responses, we investigated whether they affected expression of TNF ligands on adult inducer cells. After 2 days of incubation with the endogenous glucocorticoid cortisol or the synthetic glucocorticoid dexamethasone, inducer cells down-regulated the expression of both CD30L and TRANCE, although the expression of OX40L was only modestly attenuated (Fig. 6A). This was associated with impaired survival of inducer cells after 6 days of culture with dexamethasone (Fig. 6B). The effects of dexamethasone did not depend...
on down-regulation of IL-7Rα because levels were unaffected by treatment (Fig. 6A). Furthermore, the effects of dexamethasone on the expression of CD30L and TRANCE and their survival were prevented by addition of IL-7.

Discussion

Here we report that inducer cells isolated from neonatal mice lack expression of the T cell survival molecules, OX40L and CD30L, although expression of TRANCE and RANK was normal. We have found that mice deficient in both OX40 and CD30 show grossly impaired CD4 T cell memory responses because they fail to receive survival signals from inducer cells (14). The data provided here on neonatal inducer cells suggest that T cells primed in the neonate would be rendered effectively deficient in OX40 and CD30 survival signals from inducer cells, so they would not become memory cells. This observation may help explain the phenomenon of neonatal tolerance first described 50 years ago by Medawar et al. (20). Although neonatal mice did not express OX40L and CD30L on inducer cells, by 2 wk of age both molecules were expressed, and almost normal levels were achieved by 3 wk, the time mice were weaned. These data suggest that there must be distinct signaling pathways that regulate expression of these molecules on inducer cells after birth.

We demonstrated that IL-7 signals to inducer cells up-regulate TRANCE and CD30L expression. On adult inducer cells, CD30L expression was augmented, and on neonatal inducer cells, CD30L expression was induced by IL-7. Furthermore, mice deficient in IL-7, IL-7Rα, and γc chain had reduced CD30L expression. In contrast to CD30L, OX40L expression was not dependent on IL-7 or γc chain, and we could identify no other cytokine signals (IL-3, IL-4, IL-9, IL-10, IL-12, or IL-15) that would induce its expression on neonatal inducer cells (data not shown).

IL-7 mediated up-regulation of CD30L expression was not exclusive to inducer cells because memory CD4 and CD8 T cells also up-regulated CD30L expression in the presence of IL-7, suggesting that there is a common signaling pathway in T cells and inducer cells. We have observed previously that IL-4, which also signals via γc chain-like IL-7, does not affect the expression of either OX40L or CD30L on inducer cells (21). Its effects on CD4 T cells are different: IL-4 down-regulated OX40L and CD30L expression on primed CD4 T cells, whereas IL-7 induced CD30L but not OX40L expression. This indicates that although the cytokines, IL-4 and IL-7, both share signaling pathways through γc chain, there must be distinct signaling pathways that are γc chain independent, particularly for IL-4.

Because inducer cells express CD4, we tested whether MHC class I or II molecules might be required for their development and the expression of TNF ligands. No differences in number or phenotype were observed. Similarly the expression of these molecules was normal in mice deficient for OX40 and CD30 expression suggesting that reverse signaling through OX40L and CD30L was unimportant for inducer cell development.

Finally we investigated whether glucocorticoids, which are potent suppressors of T cell-mediated effector responses, modified the expression of TNF ligands on inducer cells. After 2 days of culture with cortisol or the more potent synthetic glucocorticoid, dexamethasone, inducer cells down-regulated both CD30L and TRANCE expression compared with control. After 6 days of culture, there were significantly fewer inducer cells in the dexamethasone-treated cultures, presumably due to down-regulation of TRANCE-dependent survival signals for inducer cells, but OX40L expression was spared. The decreased survival in the presence of glucocorticoids was reversed if inducer cells were cultured in the presence of IL-7. The failure of glucocorticoids to attenuate the expression of the T cell survival signal, OX40L explain why glucocorticoids, which are effective at suppressing effector T cell responses (22, 23), fail to eliminate CD4 T cell memory (24).

In this study, we show that OX40L and CD30L expression on adult inducer cells, which we have linked with CD4 T cell survival and memory for Ab responses, is not expressed in neonatal life on inducer cells. We provide evidence that the expression of TRANCE and CD30L on inducer cells is regulated by IL-7, γc chain, and glucocorticoid signals. In contrast, although OX40L was not expressed on neonatal inducer cells, regulation of its expression was not affected by the above signals. We suggest that the deficient expression of OX40L and CD30L on neonatal inducer cells could be a contributory mechanism for neonatal tolerance induction.

We do not know whether inducer cells isolated from adults that express OX40L and CD30L are the direct descendants of neonatal inducer cells, although labeling studies with thymidine analogues suggest that they turnover slowly (25). We have provided evidence
that CD30L expression can be induced by IL-7, but we do not know which cell provides this signal in secondary lymphoid tissue. An intriguing possibility is that it comes from follicular dendritic cells (FDCs) (26) in B follicles close to where inducer cells are found. FDCs depend on B cell LTα1β2 for their survival (27), and we have found that inducer cells isolated from neonatal mice with B cells (CD3εTg26 mice) did have higher levels of CD30L (data not shown) than when isolated from RAG-deficient mice. However, this cannot be the sole explanation, because adult inducer cells isolated from RAG-deficient mice that lack FDCs have high levels of CD30L. In any case, we have not identified the signals that regulate OX40L expression.

Inducer cells have been reported in the blood (28), and we found that in the adult they express L-selectin (11), so precursors could potentially migrate from the blood into secondary lymphoid organs. It could be that the neonatal population that we isolate is simply replaced by an adult population that expresses OX40L and CD30L. Nevertheless, understanding the molecular mechanism that regulates this transition may provide new approaches to modifying pathogenic T cell responses that are refractory to conventional immune suppression including glucocorticoid therapy.

Acknowledgments
We thank Rose Zamoyska and Benedict Seddon for providing spleens from the IL-7Rα-deficient mouse and common γ-chain-deficient RAG−/− mice.

Disclosures
The authors have no financial conflict of interest.

References